E-Article

Imperfect Morphological Convergence: Variable Changes in Cranial Structures Underlie Transitions to Durophagy in Moray Eels

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abstract: Convergence is central to the study of evolution because it demonstrates the power of natural selection to deterministically shape phenotypic diversity. However, the conditions under which a common morphology repeatedly evolves may be restrictive. Many factors, such as differing genetic and environmental backgrounds and many-to-one mapping of form to function, contribute to variability in responses to selection. Nevertheless, lineages may evolve similar, even if not identical, forms given a shared selective regime, providing opportunities to examine the relative importance of natural selection, constraint, and contingency. Here, we show that following 10 transitions to durophagy (eating hard-shelled prey) in moray eels (Muraenidae), cranial morphology repeatedly evolved toward a novel region of morphological space indicative of enhanced feeding performance on hard prey. Disparity among the resulting 15 durophagous species, however, is greater than disparity among ancestors that fed on large evasive prey, contradicting the pattern expected under convergence. This elevated disparity is a consequence of lineage-specific responses to durophagy, in which independent transitions vary in the suites of traits exhibiting the largest changes. Our results reveal a pattern of imperfect convergence, which suggests shared selection may actually promote diversification because lineages often differ in their phenotypic responses to similar selective demands.

Keywords: adaptation, functional morphology, many-to-one mapping, Muraenidae, phylogenetic comparative methods.

Introduction

Convergent evolution can illustrate the power of extrinsic selective factors to deterministically shape morphological diversity. Some environmental demands are so severe that they lead to the evolution of similar phenotypes in independent lineages despite varying biological backgrounds

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(Simpson 1953; Schluter 2000; Conway Morris 2003; Losos 2011). For example, in *Anolis* lizards, the demands of moving and perching on similar arboreal surfaces result in shared limb, body, and tail dimensions in species that evolved on separate Greater Antillean islands (Losos et al. 1998; Losos 2009; Mahler et al. 2013). In several clades of lacustrine fishes, deep-bodied, blunt-headed forms have evolved in the benthic zone, and fusiform bodies and elongated heads have arisen in limnetic species, reflecting rigorous demands of predator avoidance, locomotor efficiency, and foraging in these habitats (Robinson and Wilson 1994; Schluter 2000; Walker 1997). The clustering in morphological space of distantly related species may be more than just a consequence of strong selection toward an optimal phenotype, though, as genetics, development, functional integration, and other aspects of the environment may limit the set of possible morphological responses (Wake 1991; Conway Morris 2003, 2008; McGhee 2008). Indeed, such limits on evolution are likely necessary for convergence to occur, because even when natural selection is potent and pervasive, lineages may not be expected to reach the same morphology (Gould 2002; Donoghue 2005).

A variety of causes are thought to promote disparate morphological outcomes to a shared environmental demand. Distantly related lineages are likely to differ in many relevant intrinsic properties, such as genetic variation and functional trade-offs, as well as background environmental variables that lead to differences in their capacity to respond to selection (Arnold 1994; Schluter 1996; Hansen 1997; Gould 2002; Langerhans and DeWitt 2004; Donoghue 2005; Walker 2007). Also, species may vary in behavior—perhaps exploiting a common resource or shared habitat in different ways—and thus may experience selection on morphology differently (Stein 2000; Losos et al.

2002). Even when a selective regime imposes uniform functional demands in multiple lineages, phenotypic outcomes can vary if multiple morphological arrangements are capable of the same performance capacity (i.e., the relationship between form and function is many-to-one; Hulsey and Wainwright 2002; Alfaro et al. 2004, 2005; Garland et al. 2011). Because many-to-one mapping is likely a common property of morphological systems (Wainwright et al. 2005) and because other traits, including aspects of physiology and life history, may add to the number of phenotypic combinations that can produce equivalent fitness in a shared selective environment (Marks and Lechowicz 2006), many-to-one mapping may make convergence an unlikely outcome.

Although the conditions necessary for convergence may be restrictive, independent lineages often evolve similar, though perhaps not identical, forms when confronting a shared selective demand. But what degree of morphological similarity qualifies as convergence? Stayton (2006) offered objective criteria for evaluating this question: compared to their ancestors that experienced alternative selective regimes, convergent lineages should evolve to a distinct and restricted region of morphospace. It is possible, however, that one or more of the diversifying factors described above could result in this pattern being only partially met. For example, despite responding similarly to a shared selective regime, independent lineages may still overlap with ancestors in morphospace (i.e., they exhibit "incomplete convergence" sensu Herrel et al. 2004; Stayton 2006) or evolve to a distinct but larger region of morphospace compared to their ancestors. In this study, we document the latter pattern, which we refer to as "imperfect convergence," and we demonstrate that it can result from varying evolutionary responses among traits across independent lineages that share a selective regime. We further show how investigation into imperfect convergence can shed light on the relative importance of selection, constraints on adaptation, and lineage-specific contingency in shaping morphological diversity.

We examined morphological convergence in the skull and jaws of moray eels (Muraenidae) following transitions to a diet of hard-shelled prey (i.e., durophagy). Moray eels are a globally distributed clade of nearly 200 species of predatory marine teleost fishes (Smith 2012). The majority of morays feed on large evasive prey; their diets are composed of some combination of fish and soft-bodied cephalopods (such eels are referred to throughout as "piscivores"). But approximately 10%–15% of species are known to consume hard-shelled prey, such as snails, bivalves, and crabs (Böhlke et al. 1989). The phylogenetic distribution of durophagy is uncertain, but the condition has evolved independently at least four times across two recognized genera, *Gymnomuraena* and *Echidna* (Reece et al. 2010).

Multiple origins of durophagy in morays provide an opportunity to examine the degree of convergence under the shared demands of crushing hard prey and to test the roles of selection and contingency on evolutionary responses to this diet.

The prevailing functional demand for eating hard-bodied prey is the generation of a forceful bite to crush the external shell or carapace so that the prey may be extracted. In a variety of durophagous vertebrate taxa—including canids (Tseng and Wang 2011), bats (Nogueira et al. 2005), squamates (Dalrymple 1979; Savitzky 1983; Pregill 1984), sharks (Summers 2000; Huber et al. 2008; Mara et al. 2010), and ray-finned fishes (Galis and Drucker 1996; Grubich 2003; Wainwright et al. 2012)—a similar and predictable suite of morphological adaptations has evolved to increase bite force and crushing ability. In particular, durophagous teleost fishes typically use their pharyngeal jaws—modified branchial arches just anterior to the esophagus—to crush the shells of their prey, and these species almost universally show enlarged pharyngeal tooth plates, hypertrophied adductor muscles, and blunt teeth compared to their nondurophagous relatives (Lauder 1983; Galis and Drucker 1996; Hulsey et al. 2010).

Unlike most other teleost fishes, moray eels use their oral jaws for both prey capture and processing because their pharyngeal jaws are specialized for prey transport. Whereas most teleosts use suction-induced water motion to draw prey into the mouth and transport it through the oral cavity to the pharyngeal jaws, morays apprehend their prey by biting and then protract their raptorial pharyngeal jaws from behind the head into the oral cavity to grip prey and carry it into the esophagus (Mehta and Wainwright 2007, 2008). In morays, the pharyngeal jaw tooth plates are lined with sharp, recurved teeth and move mainly forward and backward, making them unsuitable for the processing functions that pharyngeal jaws serve in other teleosts (Mehta and Wainwright 2008; Mehta 2009). To crush hard prey, morays must therefore generate large bite forces in their oral jaws. This derived mechanism of crushing prey makes moray eels a compelling case study for examining convergence. Do durophagous morays consistently evolve robust oral jaw bones, strong adductor muscles, and blunt teeth as seen in the pharyngeal jaws of other teleosts that share this diet, or is this distinctive crushing mechanism associated with varied morphological responses?

In this study, we combined information on moray eel diets, measurements of functionally important morphological traits of the skull, and phylogenetic reconstruction for a sample of 40 moray species to test the hypothesis that durophagous morays have undergone convergent morphological evolution. We identified a pattern of imperfect convergence in which durophagous morays follow

similar trajectories to a novel region of morphological space but exhibit greater disparity than their piscivorous ancestors. We assessed whether this unexpectedly elevated disparity is a result of evolution toward multiple adaptive peaks for durophagy associated with consumption of different types of hard-shelled prey. Finding little support for this explanation, we demonstrate that imperfect convergence is a consequence of variability among individual traits in their responses to durophagy across moray lineages.

Methods

Sampling and Morphological Measurements

We obtained 176 individuals representing 40 moray species (median number of individuals per species = 3, range = [1, 8]) from museums or local aquarium stores (see table A1; tables A1–A6 available online). We recorded body mass for each specimen, and to visualize internal skeletal anatomy, we cleared and stained specimens following standard methods (Dingerkus and Uhler 1977; Song and Parenti 1995). Morphological measurements (described below) for species are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.h9r37 (Collar et al. 2014).

We measured five features of cranial musculoskeletal anatomy that have predictable consequences for feeding performance on hard-shelled prey. (1) The adductor mandibulae (AM) muscle is the primary muscle powering jaw adduction, and we measured its mass to reflect its capacity to generate force and motion in the lower jaw during biting (Alfaro et al. 2001; Westneat 2003). Although physiological cross sectional area (PCSA) of the AM muscle would be a more direct measure of its force capacity, we measured its mass because this variable is more readily measured for large numbers of specimens, particularly when the AM is composed of multiple subdivisions as it is in morays. Muscle mass should reflect species differences in muscle force capacity as long as other muscle properties, such as fiber lengths, remain relatively constant. In fact, AM mass and PCSA are tightly correlated for a subset of species in our sample ($r = 0.95$; $P < .001$). We therefore expect AM mass to increase during transitions to durophagy to increase biting force. (2) Lower jaw closing mechanical advantage (MA) is the ratio of the in-lever for jaw closing to its out-lever and describes the proportion of AM muscle force transmitted to the jaw tip (Barel 1983; Westneat 1994, 2004). Jaw closing MA is expected to increase in durophagous morays because larger MA translates to greater bite force. (3) Maxilla length is the distance between the scarf joint attaching it to the fused premaxillo-ethmo-vomer medially and its distal tip. While the maxilla bears teeth that aid in apprehension of evasive prey, it is expected

to shorten in durophagous species to accommodate lateral expansion of the AM as it increases in cross-sectional area and force capacity. (4) Premaxillo-ethmo-vomerine (PMx-Et-V) area is the product of its antero-posterior length and lateral width. This tooth-bearing bone makes up the roof of the mouth cavity and is the dorsal surface against which hard prey are compressed during biting. We expect PMx-Et-V area to increase with transitions to durophagy to provide a larger contact area for crushing prey. (5) Supraoccipital (SO) length describes the extent to which this bone extends dorsally and posteriorly from the neurocranium, and it is expected to become elongated in durophagous morays to provide additional attachment area for the enlarged AM muscle.

In addition, we measured dimensions of the teeth on the PMx-Et-V and maxilla. Tooth length is the vertical distance from the base of the tooth to its tip, and tooth width is the lateral distance at its base. These measurements were made on the single longest tooth on the PMx-Et-V, but for the maxillary teeth, these dimensions were taken as the means of measurements made on three teeth, which were sampled from the anterior, posterior, and middle portions of the maxilla. We expected teeth to become shorter and wider, reflecting a shift from pointed conical structures to blunt molariform teeth as morays become durophagous.

We evaluated species means for each variable and log-transformed these values. We then accounted for variation in trait values that result from body size differences by obtaining residuals from phylogenetic regression of each trait on body mass following the procedure of Revell (2009), which is implemented in the function phyl.resid in the package phytools (Revell 2012) for the R statistical computing environment (R Core Development Team 2013). We note that because the effectiveness of clearing and staining decreases with increasing specimen size, we sampled a relatively narrow range of body sizes. The specimens of piscivorous and durophagous species in our sample overlapped broadly in body mass (piscivore median = 10.0 g, piscivore range = [1.7 g, 78.8 g]; durophagy median = 12.3 g, durophagy range = [1.9 g, 119.3 g]), allaying concerns that differences in body size between diet groups could cause the regression to remove shape variation associated with diet.

Assigning Diet States to Moray Species

We conducted an intensive survey of published diet studies in moray eels and assigned diet states—durophagous or piscivorous—to 43 species in our phylogenetic analysis (see below). Based on this analysis, we determined that durophagy and piscivory are dichotomous states; volumetric proportions of hard prey in species' diets reveal

moray species primarily consume one prey type or the other (see fig. A1; table A2). Durophagous species' diets are made up of more than 70% hard prey, including shelled molluscs, crustaceans, and urchins. Piscivores, on the other hand, consume only very small proportions of hard prey; instead, more than 70% of their diets are made up of soft-bodied prey items—mainly fish and cephalopods. Published diet information was unavailable for six of the sampled species (*Uropterygius fuscoguttatus* and *Gymnothorax* species *G. chilospilus*, *G. minor*, *G. richardsoni*, *G. robinsi*, and *G. zonipectis*), but we were able to classify each of these species as piscivores based on discriminant function analysis of a suite of morphological traits that differentiate piscivorous and durophagous species but that do not overlap with the variables in our main analysis (see "Supplementary Methods," available online).

Reconstructing the Evolutionary History of Diet

To identify independent transitions to durophagy, we estimated phylogenetic relationships among 46 moray species and used this information in combination with observed diets for species to reconstruct ancestral diets. Phylogenetic analysis was performed on DNA sequences of two mitochondrial (1.2 kb) and two nuclear (1.2 kb) gene regions from 44 species included in a previous molecular phylogeny (Reece et al. 2010) as well as two new moray species, *Echidna catenata* and *Pseudoechidna brummeri* (GenBank accession numbers 1442581 and 1442588–90). Sequencing and Bayesian phylogenetic analysis in BEAST (Drummond et al. 2006; Drummond and Rambaut 2007) followed Reece et al. (2010) and are described in "Supplementary Methods." The resulting maximum clade credibility tree was well resolved with nearly all nodes highly supported ($\geq$.90 Bayesian posterior probabilities; see fig. 1; sequence alignment and tree are available in TreeBase: study accession number S11357). Subsequent analyses used this tree, which was pruned of three *Gymnothorax* species (*G. albimarginatus*, *G. pseduthrysoideus*, and *G. reevesii*) for which neither diet information nor morphological data could be obtained.

We then applied stochastic character mapping (Nielsen 2002; Huelsenbeck et al. 2003) using the function make.simmap of the phytools package (Revell 2012) for R to infer the history of piscivory and durophagy given observed diet states in moray species and the maximum clade credibility phylogeny. We used fixed, empirically determined priors for transition rates to and from durophagy and for the stationary distribution of states (see "Supplementary Methods") and sampled 1,000 stochastic maps of dietary history in proportion to their posterior probabilities. We used the phytools function describe.simmap to calculate the Bayesian posterior probabilities (BPP) of pis-

civory at each node on the moray phylogeny. We identified transitions to durophagy as the branches across which BPP of piscivory changed by more than .50 from ancestral to descendant nodes (i.e., branches on which piscivory was the favored state in the ancestor but durophagy was favored in the descendant).

Comparing Disparity in Durophagous Morays to Their Piscivorous Ancestors

Following Stayton's (2006) criteria for evaluating convergence using multivariate morphological data, we assessed whether durophagous species occupy a region of morphospace that is both distinct from and smaller than the area occupied by their piscivorous ancestors. We first constructed a morphospace of log-transformed, size-corrected cranial morphological traits for 40 species (15 durophagous and 25 piscivorous species). We summarized the diversity of moray eels on a reduced set of morphological axes using phylogenetically controlled principal components analysis implemented in the phytools function phyl.pca (Revell 2012). We retained significant principal components (PCs), assessed using a broken-stick distribution of variances (Legendre and Legendre 1998), and quantified morphological disparity within durophagy as the variance among species scores for these axes (Foote 1997).

To estimate disparity of the piscivorous ancestors that gave rise to durophagous morays, we calculated the variance in PC scores reconstructed for the nodes immediately preceding each transition to durophagy (referred to hereafter as the most recent piscivorous ancestors, or MRPAs, to durophagous transitions). We reconstructed ancestral states given species PC scores and the moray phylogeny, which had been transformed according to the empirical estimate of the piscivore-specific strength of selection parameter (α) from an Ornstein-Uhlenbeck (OU) model (see "Supplementary Methods"). We then assessed the significance of disparity differences between durophagous species and their MRPAs separately on PCs 1 and 2 using Levene's heterogeneity of variance test (Brown and Forsythe 1974).

Comparing Variation in Evolutionary Trajectories

Examination of morphospace (PCs 1 and 2) revealed that durophagous species and their piscivorous ancestors occupy nonoverlapping regions, but durophagous morays exhibit greater disparity on these axes (see "Results"). Although elevated disparity is inconsistent with the strictest definitions of convergent evolution, this pattern could be the result of similar responses to shared demands of crushing hard prey if durophagous species have followed similar
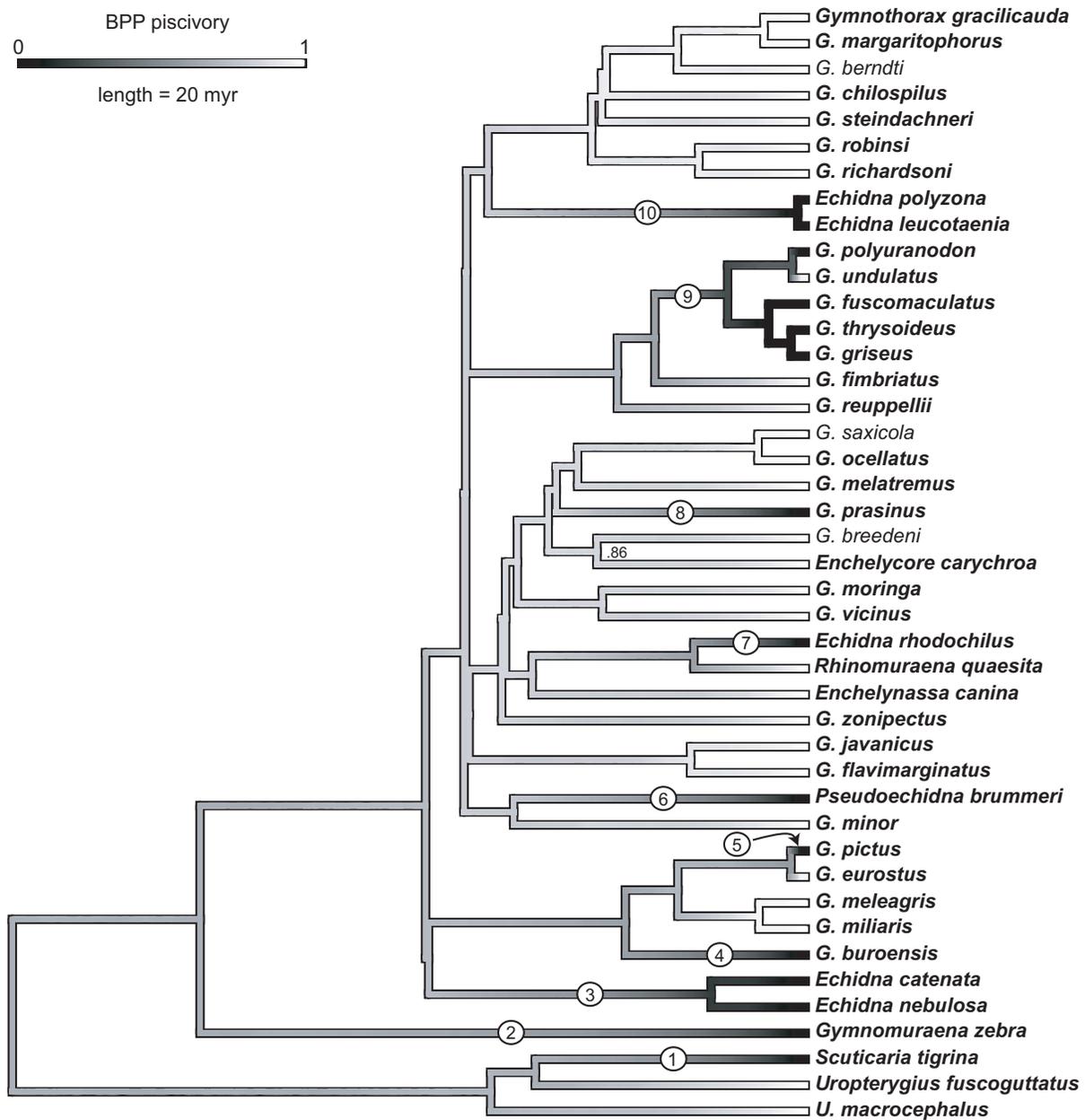
Figure 1: Summary of stochastic diet maps on the maximum clade credibility phylogeny for 43 moray eel species. Branch lengths are given in millions of years (My); scale bar shown in the upper left inset. Nodes receive 0.90 or greater Bayesian posterior probability (BPP) support unless otherwise indicated. Species names in bold are those included in our morphological data set. Shading on branches represents BPP of piscivory for branch segments across 1,000 stochastic character maps. White indicates that the BPP of piscivory equals 1, and darker shading corresponds with lower BPP of piscivory and therefore higher BPP of durophagy. We inferred 10 durophagous transitions, identified by encircled numbers. Transitions were determined to occur along branches on which ancestral nodes are light gray (favoring piscivory), and descendant nodes are dark gray or black (favoring durophagy). Note that the positioning of numbers along branches is arbitrary and not meant to suggest specific timing of transitions. This figure was generated using the phytools function densityMap (Revell 2012, 2013).

paths through morphospace to their current positions (Gould 2002; Stayton 2006; Losos 2011). To evaluate this possibility, we assessed the degree of similarity among durophagous species in their evolutionary trajectories using the method of Collyer and Adams (2007; also described in Adams and Collyer 2007, 2009). Evolutionary trajectories follow the path of multivariate morphological change associated with shifts to durophagy and are the

vectors extending from the inferred state for the MRPAs to the derived state in the resulting durophagous species. Note that for transitions leading to multiple durophagous species, the derived state was the median for those species.

To evaluate the degree of similarity among morays in response to durophagy, we tested whether the direction of evolutionary trajectories associated with transitions to durophagy are more similar to one another than trajectories leading to piscivores. These latter trajectories connect each piscivorous species to the ancestral state inferred at the shallowest node subtending it. We excluded the piscivorous species, *Gymnothorax undulatus*, which likely experienced a secondary transition to piscivory (see fig. 1), so that these evolutionary trajectories for piscivores represent morphological evolution within morays that is not associated with dietary transitions. They therefore provide a suitable background distribution of evolutionary changes against which to compare durophagous trajectories. We quantified direction (in radians) of each evolutionary trajectory in the morphospace defined by PCs 1 and 2 following Collyer and Adams (2007). We then compared the variance of directions for durophagous and piscivorous trajectories using Levene's test. Significantly lower variance among durophagous trajectories is evidence of similarity among lineages in their responses to the demands of durophagy.

Testing Multiple Durophagous Adaptive Peaks

Heterogeneity in the diets of durophagous morays may contribute to their unexpectedly elevated morphological diversity, as species differ in the amount and type of hard-shelled prey they consume. In particular, we identified four highly durophagous moray species—*Gymnomuraena zebra* and *Echidna* species *E. leucotaenia*, *E. polyzona*, and *E. catenata*—that feed almost exclusively on hard-shelled prey (≥90% of the volumetric content of their diets; see table A2). In addition, the diet of *G. zebra* includes a substantial proportion of bivalves and urchins, the hardest prey items of the durophagous moray diet (Hiatt and Strasburg 1960; Hobson 1974). In other durophagous species, hard-shelled prey makes up a smaller proportion of the diet and their prey tend to be relatively thin-shelled (e.g., xanthid crabs) or of intermediate hardness (e.g., some gastropod taxa). Because these categories (highly durophagous vs. moderately durophagous) may differ in their demands on feeding performance, they could impose selection for different skull and jaw morphologies.

To evaluate this possibility, we fit OU models that allowed optimum positions (θ) and stochastic evolutionary rates (σ^2) for PCs 1 and 2 to vary among lineages inferred to be piscivorous, moderately durophagous, and highly durophagous (see Beaulieu et al. 2012). We specified four

OU models with separate diet-associated optima: (1) a three-θ, three-σ^2 model in which optima positions and stochastic evolutionary rates are allowed to vary among diet categories; (2) a three-θ, one-σ^2 model in which the three diet categories have separate optima but share the same stochastic rate; (3) a two-θ, two-σ^2 model in which all durophagous morays share the same optimum and stochastic rate, but these are allowed to differ from those of piscivores; and (4) a two-θ, one-σ^2 model, which specifies separate optima in piscivores and durophagous species, but all morays share the same stochastic rate. In addition, we specified two null models that assume model parameters to be equal in all morays regardless of diet state: an OU model with a single θ and σ^2 and a Brownian motion model with a single σ^2. We note that all models specified a single strength of selection (α) shared by all moray lineages because allowing α to vary among diet states led to unreliable parameter estimation (i.e., negative estimation variance indicating a saddle point on the likelihood surface; see Beaulieu et al. 2012).

We used maximum likelihood implemented in the R package OUwie (Beaulieu et al. 2012) to fit each model to PCs 1 and 2. Because this analysis included only the subset of species for which published diet information was available ($n = 31$ species; see table A2) and included a third diet category (i.e., highly piscivorous), we generated an additional set of stochastic diet maps on the maximum clade credibility phylogeny using the methods described above. We quantified model fit using the small sample size corrected Akaike Information Criterion (AICc; Burnham and Anderson 2002) and performed model selection based on the distributions of differences in AICc scores among models (ΔAICc, which equals a model's AICc minus the minimum AICc among models) across stochastic diet maps. We then compared diet-associated optima from the best-fit models to evaluate whether differences in durophagous diets contribute to variation among durophagous morays. In addition, we assessed the overall responsiveness of moray lineages to diet-imposed selective regimes, which we quantified as phylogenetic half-life ($= \ln (2)/\alpha$). This parameter describes the amount of time (in units of branch length) required to traverse half the morphological distance from the ancestral state to the optima (Hansen 1997; Hansen and Orzack 2005). A phylogenetic half-life that is long relative to the total depth of the tree indicates slow evolution toward diet-associated optima and can contribute to morphological diversity among species that share a selective regime when they have evolved under that regime for different amounts of time (Hansen 1997).

Examining Responses of Individual Traits to Durophagy

Because some traits may be freer than others to evolve in response to selection imposed by the demands of duro-

phagy, we also evaluated convergence in individual morphological traits. For each of the nine traits in our data set, we conducted a modified version of the test of similarity in evolutionary trajectories (Adams and Collyer 2007; Collyer and Adams 2007). We reconstructed individual trait trajectories associated with transitions to durophagy as the vectors extending between inferred ancestral states at MRPAs and trait values for durophagous species. However, because individual traits can change in only a positive or negative direction, we tested for exceptional similarity in trajectory directions among durophagous lineages using a binomial test, in which the number of durophagous transitions exhibiting trajectories in the direction expected for increasing performance on hard prey (e.g., increasing lower jaw MA) served as our test statistic. We then evaluated the probability of observing an equal or greater number of changes in that direction given a binomial distribution with number of observations (n) equal to the number of durophagous transitions and probability of success (p) equal to the proportion of changes in that direction observed for piscivorous trajectories. We concluded that a trait exhibits convergence when this probability is less than .05.

We also assessed lineage-specific morphological responses to durophagy. We identified traits within each independent durophagous transition whose evolutionary trajectories—both direction and magnitude—differed significantly from trajectories of piscivorous lineages. For each durophagous transition and each individual trait, we standardized trajectories by the mean and standard deviation estimated for 24 trajectories leading to piscivorous species and evaluated the probability of observing each durophagous trajectory given Student's t-distribution with 22 degrees of freedom. Trajectories with probability less than .05 were considered significant deviations from the background pattern of evolution in piscivores and were therefore interpreted as exceptional morphological responses to durophagy. We then examined overlap among lineages in the traits showing exceptional responses to durophagy.

One simple explanation for variation in evolutionary trajectories among durophagous transitions is that the amount of trait evolution is related to the morphology of the MRPA. Some lineages may experience little trait evolution during the transition to durophagy if their piscivorous ancestors possessed trait values that are well suited to crushing hard prey (e.g., they already possessed relatively large AM muscles), which should result in a negative association between durophagous trajectories (direction and magnitude) and reconstructed states in MRPAs. To test for this relationship we estimated Pearson's correlation coefficient for each trait and evaluated the probability that it differs from zero.

Results

Based on stochastic character mapping, we inferred 10 independent transitions to durophagy within moray eels (fig. 1). Change in Bayesian posterior probability (BPP) of piscivory was greater than .55 for all but one of the inferred transition branches; the shift to durophagy on the branch subtending the clade containing the durophagous *Gymnothorax* species *G. fuscomaculatus*, *G. griseus*, *G. polyuranodon*, and *G. thrysoideus* (transition 9 in fig. 1, referred to hereafter as *Gymnothorax* group 1) had a BPP change of .38, but this change was much larger than those estimated for deeper branches. In fact, changes in BPP of piscivory for all 10 transition branches were greater than those estimated for any of the other branches on the phylogeny (see fig. A2). We note that BPPs of piscivory at the deepest nodes of the moray phylogeny indicate uncertainty in ancestral diet state (fig. 1). We suggest, however, that the ancestral moray was likely piscivorous, and the support we found for this reconstruction is likely biased downward because durophagous species are overrepresented in our sample compared to the actual proportion of moray species that are durophagous; 37.5% of species in our data set are durophagous, but only 10%–15% of recognized moray species are thought to feed extensively on hard-shelled prey (Böhlke et al. 1989). This consideration in combination with the changes in BPP of piscivory described above support our inference of 10 independent transitions to durophagy from piscivorous ancestors.

Principal components analysis on log-transformed, size-corrected morphological traits revealed two significant axes that together account for 61% of the total morphological variation among moray eels (fig. 2; table A3). Principal component 1 almost completely separates durophagous and piscivorous morays, indicating that this diet split has strongly influenced the primary axis of cranial diversification in moray eels (fig. 2). All but three durophagous species exhibit PC 1 scores less than those of any piscivore, and the remaining durophagous species exceed all but the piscivorous *Gymnothorax melatremus*. The distribution of species on this axis reveals that durophagous species generally have shorter maxillae, smaller teeth on the maxilla and PMx-Et-V, greater MA for lower jaw closing, and elongated supraoccipitals. Durophagous and piscivorous morays overlap broadly on PC 2, which loads positively with PMx-Et-V area, supraoccipital length, AM mass, and width of maxillary and PMx-Et-V teeth.

Contrary to the pattern expected under convergence, durophagous morays do not exhibit reduced morphological disparity compared to their most recent piscivorous ancestors (MRPAs). Instead, variance among durophagous species on PCs 1 and 2 is actually significantly greater by Levene's test (PC 1: Levene's $W = 9.10$, $P = .007$; PC 2:
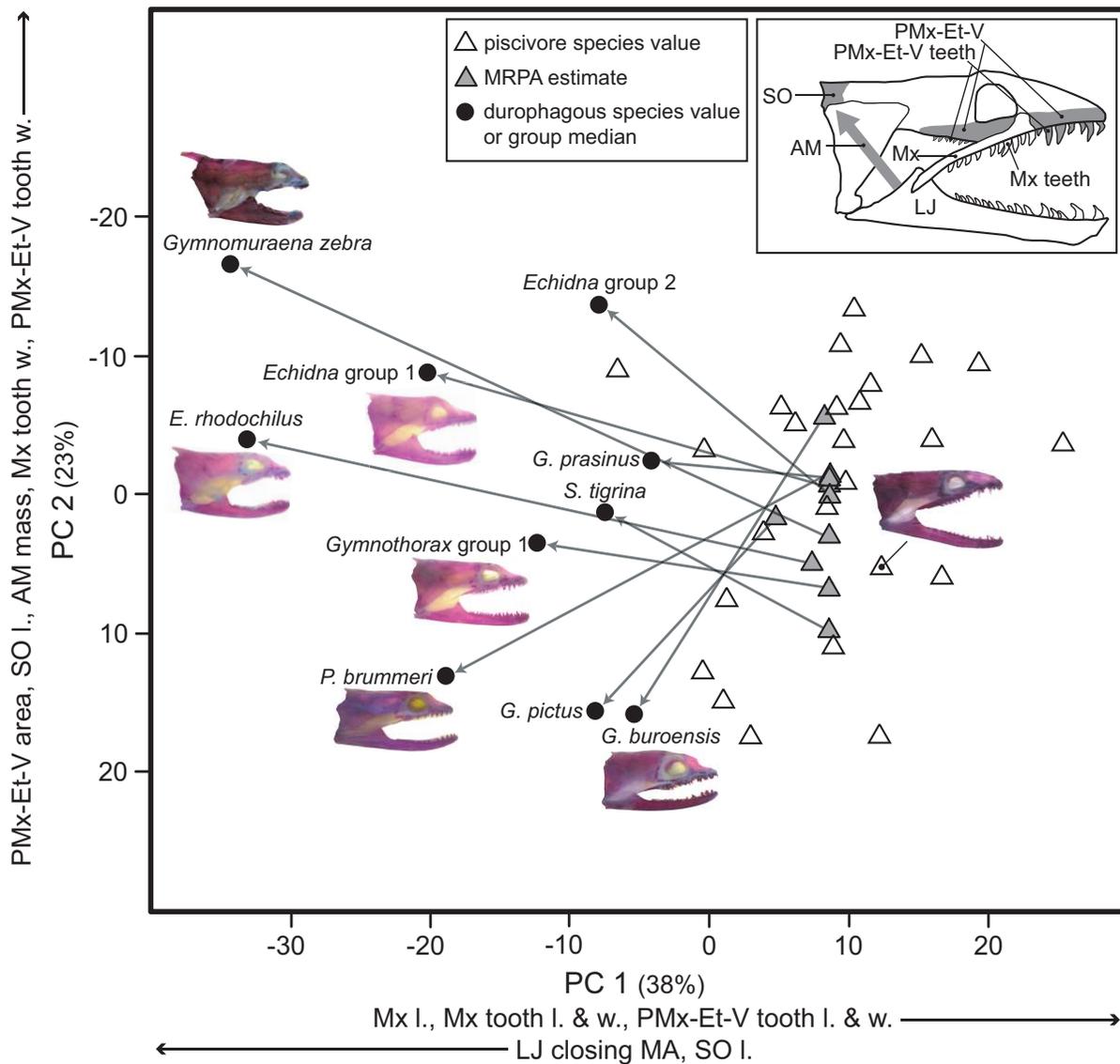
Figure 2: Morphospace for moray eels highlighting evolutionary trajectories for transitions to durophagy. Axes are principal components 1 and 2 of a phylogenetically controlled principal component analysis on the correlation matrix of log-transformed, size-corrected morphological traits for 40 moray eel species. Axis labels give the percent of total morphological variation explained as well as loadings on individual morphological traits. Inset illustrates morphological traits on a piscivorous moray eel skull in lateral view. White triangles are scores for piscivorous species. Gray triangles are estimated scores for the most recent piscivorous ancestors (MRPAs) leading to durophagous transitions. Black circles are durophagous species scores when transitions lead to a single species or median scores for groups of durophagous species that share a transition (see fig. 1). There are three such groups: *Echidna* group 1 (*E. catenata, E. nebulosa*), *Echidna* group 2 (*E. leucotaenia* and *E. polyzona*), and *Gymnothorax* group 1 (*G. fuscomaculatus, G. griseus, G. polyuranodon*, and *G. thrysoideus*). Skull images are cleared and stained neurocrania and oral jaws for a subsample of durophagous species (*Gymnothorax* group 1 is represented by *G. polyuranodon* and *Echidna* group 1 is represented by *E. nebulosa*) and one piscivore (*Enchelycore carychoa*). Arrows extending from MRPAs to durophagous descendants are evolutionary trajectories through morphospace. AM = adductor mandibulae, LJ = lower jaw, MA = mechanical advantage, Mx = maxilla, PMx-Et-V = premaxillo-ethmo-vomerine, and SO = supraoccipital; l. = length, w. = width.

Levene's $W = 7.70$, $P = .013$; see fig. 2). This result is partially a consequence of estimating ancestral states as intermediates of extant taxa, but we also found that variances on PCs 1 and 2 were about twofold greater among durophagous species than among piscivores, though these differences are nonsignificant (PC 1: Levene's $W = 0.80$, $P = .378$; PC 2: Levene's $W = 1.88$, $P = .179$; see fig. 2).

Reconstructions of evolutionary trajectories in mor-

phospace (PCs 1 and 2) revealed that morphological changes associated with the adoption of durophagy are more similar to one another in direction than are changes within piscivorous lineages (Levene's $W = 18.21$, $P < .001$; see figs. 2, 3). All trajectories associated with durophagous transitions point toward negative values on PC 1, and 7 of 10 point toward positive values on PC 2. In contrast, evolutionary trajectories leading to piscivorous species are nearly uniformly distributed in all directions (fig. 3).

We found little support for the hypothesis that variation among durophagous morays is driven by evolution toward separate morphological optima corresponding to moderately and highly durophagous diets. For PC 1, the two models inferring separate optima for the two categories of durophagy combine to receive only 2.2% of the available AICc support on average and are disfavored (ΔAICc > 4) for more than 70% of stochastic diet maps (table 1). Instead, the best-fit model for PC 1 is the OU model in which durophagous and piscivorous morays differ in optimum and rate but all durophagous lineages share the same values for these parameters (table 1). The strength of selection inferred for this model is moderate ($\alpha = 0.078$ per million years (myr), 95% confidence interval (CI) = [0.018, 0.138] myr^{-1}), corresponding to a phylogenetic half-life equal to 8.9 myr (95% CI = [5.0, 38.7] myr), which is about half the mean time since durophagous lineages split from their MRPAs (18.4 myr). This relatively long phylogenetic half-life indicates that lineage-specific factors (e.g., genetics, environmental backgrounds) resisted adaptation and that variation among durophagous morays is partly a result of relatively slow adaptive evolution and differences in the amount of time spent evolving under this regime (see Hansen 1997).

For PC 2, AICc support is split between the OU model with two optima and two stochastic rates (one each for durophagy and piscivory) and the OU model that further allowed optima and rates to differ between the two categories of durophagy (table 1). Neither of these models, however, supports distinct optima for different diet categories, as confidence intervals for optima positions on PC 2 overlap broadly (two-peak OU: $\theta_{\text{pisc}} = -1.6$, 95% CI = $[-7.1, 4.0]$; $\theta_{\text{duro}} = 4.3$, 95% CI = $[-12.9, 21.5]$; three-peak OU: $\theta_{\text{pisc}} = -5.3$, 95% CI = $[-14.6, 4.0]$; $\theta_{\text{duro}} = -31.1$, 95% CI = $[-149.1, 86.8]$; $\theta_{\text{highly duro}} = 4.3$, 95% CI = $[-13.5, 91.0]$; note that 95% confidence intervals account for error in parameter estimation and uncertainty in ancestral diet reconstruction). Accordingly, these models infer weak selection; the Akaike-weighted mean estimate of selection is negligible ($\alpha = 1.0 \times 10^{-6}$ myr^{-1}, 95% CI = $[1.0 \times 10^{-6}, 0.028]$ myr^{-1}) and even the lower 95% confidence bound for phylogenetic half-life exceeds the time to the MRPA for all but two durophagous transitions (phylogenetic half-life = 6.9×10^{5} myr, 95% CI = $[25.1, 6.9 \times 10^{5}]$ myr).

We found evidence of significant convergence in response to durophagy for three of the nine measured cranial morphological traits; maxilla length, maxilla tooth length, and PMx-Et-V tooth length all shortened in 10 of 10 durophagous transitions (maxilla length: $P[X \geq 10 \mid n = 10, p = .54] = .0022$; maxilla tooth length: $P[X \geq 10 \mid n = 10, p = .58] = .0046$; PMx-Et-V tooth length: $P[X \geq 10 \mid n = 10, p = .46] = .0004$). Three other traits
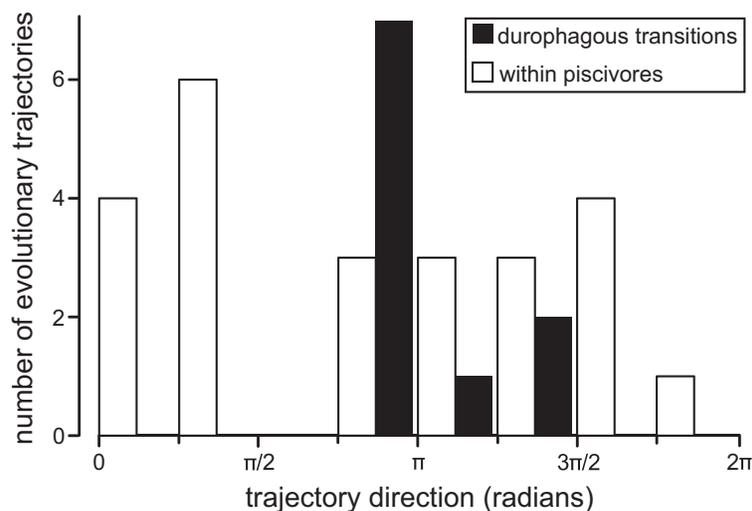

Figure 3: Histogram of orientations (in radians) for 10 evolutionary trajectories associated with transitions to durophagy (black bars) and 24 trajectories leading to piscivorous species (white bars). Durophagous trajectory directions are significantly more similar to one another than are piscivorous trajectories by Levene's test (see "Results").

Table 1: Model selection for evolutionary models fitted to principal components (PCs) 1 and 2 given 1,000 stochastic diet maps on the moray phylogeny

Trait, model	k	$-\log L \pm$ SE	AICc $\pm$ SE	ΔAICc (mid-95% CI)	AICw (mid-95% CI)	Prop pref	Prop disf
PC 1:							
BM1	2	$-128.20 \pm .00$	$260.83 \pm .00$	13.40 (2.43, 23.16)	.001 (.00, .14)	.02	.85
OU1	3	$-128.20 \pm .00$	$263.29 \pm .00$	15.85 (4.89, 25.62)	.000 (.00, .04)	.00	.94
OU2M	4	-124.38 ± 2.55	258.29 ± 5.09	10.86 (.00, 20.55)	.004 (.00, .34)	.11	.78
OU2MV	5	-117.52 ± 4.19	247.44 ± 8.39	.00 (.00, 2.83)	.973 (.11, 1.00)	.77	.05
OU3M	5	-123.76 ± 3.57	259.92 ± 7.14	12.48 (2.77, 22.54)	.002 (.00, .11)	.03	.84
OU3MV	7	-118.19 ± 4.09	255.24 ± 8.18	7.80 (.34, 18.99)	.020 (.00, .37)	.07	.72
PC 2:							
BM1	2	$-133.00 \pm .00$	$270.42 \pm .00$	12.80 (11.50, 23.47)	.001 (.00, .01)	.01	.98
OU1	3	$-133.00 \pm .00$	$272.88 \pm .00$	15.26 (13.96, 25.93)	.000 (.00, .00)	.00	.99
OU2M	4	$-132.93 \pm .08$	$275.40 \pm .15$	17.78 (16.54, 28.45)	.000 (.00, .00)	.00	1.00
OU2MV	5	-122.61 ± 4.02	257.62 ± 8.05	.00 (.00, 13.54)	.691 (.00, 1.00)	.44	.37
OU3M	5	$-132.45 \pm .30$	$277.29 \pm .60$	19.70 (18.57, 30.38)	.000 (.00, .00)	.00	1.00
OU3MV	7	-120.19 ± 4.87	259.25 ± 9.74	1.62 (.00, 24.52)	.307 (.00, 1.00)	.54	.30

Note: Models: BM1 is Brownian motion with ancestral state (θ_0) and one rate (σ^2); OU1 is an Ornstein-Uhlenbeck process with one strength of selection (α), one optimum (θ_0), and one stochastic rate (σ^2); OU2M is OU with one α, two optima (θ_{pisc} and θ_{duro}), and one σ^2; OU2MV is OU with one α, two optima (θ_{pisc} and θ_{duro}), and two stochastic rates (σ^2_{pisc} and σ^2_{duro}); OU3M is OU with one α, three optima (θ_{pisc}, θ_{duro}, $\theta_{\text{highly duro}}$), and one σ^2; OU3MV is OU with one α, three optima (θ_{pisc}, θ_{duro}, $\theta_{\text{highly duro}}$), and three stochastic rates (σ^2_{pisc}, σ^2_{duro}, $\sigma^2_{\text{highly duro}}$). Prop pref is the proportion of stochastic diet maps on which the model was preferred (i.e., it had the lowest corrected Akaike Information Criterion [AICc]). Prop disf is the proportion of stochastic diet maps on which the model was disfavored (i.e., ΔAICc > 4). AICw = weighted AIC. CI = confidence interval.

show some evidence of convergence, though similarity in direction was marginally nonsignificant (probably because of low power stemming from a relatively small n ($=10$ transitions) and p derived from the frequency of change in the expected direction in piscivores, which was sometimes greater than .5); lower jaw MA increased in nine transitions ($P[X \geq 9 \mid n = 10, p = .63] = .064$), AM mass increased in eight ($P[X \geq 8 \mid n = 10, p = .58] = .14$), and supraoccipital length increased in seven ($P[X \geq 7 \mid n = 10, p = .46] = .11$). Three traits do not exhibit convergence; PMx-Et-V area increased in only five durophagous transitions ($P[X \geq 5 \mid n = 10, p = .46] = .51$), and widths of maxillary and PMx-Et-V teeth each increased in only two lineages (maxillary tooth width: $P[X \geq 2 \mid n = 10, p = .42] = .96$; PMx-Et-V tooth width: $P[X \geq 2 \mid n = 10, p = .54] = .99$). The latter two results cast doubt on the expected relationship between tooth width and feeding performance on hard prey, as teeth consistently become smaller, decreasing in both length and width, during durophagous transitions.

The 10 independent durophagous transitions varied in the extent of morphological evolution underlying the diet shift (fig. 4; table A4). Although no durophagous transition experienced changes in the expected direction for all nine morphological traits, this result is a consequence of tooth widths rarely changing in the expected direction (see above). For simplicity, we exclude maxillary and PMx-Et-V tooth widths from figure 4 and from this discussion.

We found three durophagous transitions—the one shared by *Echidna catenata* and *Echidna nebulosa* (referred to as *Echidna* group 1) and those leading to *Gymnomuraena zebra* and *Echidna rhodochilus*—involve changes in the expected direction for all seven of the remaining cranial traits. In the other transitions, no fewer than five traits change in the expected direction. However, even though lineages exhibit similar numbers of traits responding to selection in the same direction, they vary in the morphological features that experienced the greatest magnitudes of change. All 10 transitions differ in the combination of traits that show significantly greater magnitudes of change than the background changes estimated within piscivores (fig. 4). *Echidna* group 1 exhibits the most dramatic cranial change—seven traits experienced exceptional changes. On the other extreme, *Gymnothorax buroensis* has experienced the weakest response to durophagy with only maxillary tooth length showing a significantly greater magnitude of change than piscivores. The remaining durophagous transitions involve three to six traits whose trajectories are significantly different than piscivores, though the exact suite of traits differs among lineages (fig. 4).

Variation in trajectories for individual traits does not seem to be strongly related to variation in ancestral states prior to diet transitions. Correlations between durophagous trajectories and estimated MRPA values are generally low and nonsignificant across traits (table 2). Only lower jaw MA exhibits a strong negative correlation between

Figure 4: Summary of individual morphological trait trajectories for each of 10 durophagous transitions. Colors indicate trajectory direction and significance compared to trajectories within piscivores: gray represents change in the opposite direction expected for increasing durophagous performance, yellow is change in the expected direction that does not differ significantly from piscivores, and red indicates change in the expected direction that is significantly different ($P < .05$) from piscivores. Colors on maxillary teeth and PMx-Et-V teeth correspond to the trajectories for their lengths (i.e., trajectories for tooth widths are not shown). Skulls for independent durophagous transitions are ordered along the X-axis according to the number of traits changing in the expected direction.

trajectories and ancestral states. Although the correlation is also significant for supraoccipital length, it is in the positive direction—large increases in this trait are linked to ancestors with supraoccipitals that were already long.

Discussion

In this study, we document a case of imperfect morphological convergence; repeated transitions to a functionally demanding diet led independent moray lineages to evolve into a distinct region of morphospace, but these species exhibit greater disparity than either their ancestors or closely related species that feed on other prey (fig. 2). Although this pattern does not conform to the condition of derived similarity among lineages, which is necessary to consider it an example of convergence, evolution through morphospace along similarly oriented trajectories indicates shared response to selection. We agree with others

who have argued that this pattern is consistent with the fundamental process of convergent evolution (see Stayton 2006; Losos 2011). While it is tempting to describe similarity in evolutionary trajectories as parallel evolution, we avoid this term because it is often applied to indicate similarity in both phenotypic response and the genetic and developmental changes underlying it (Wake 1991; Arendt and Reznick 2007). Differences among lineages in the exact suite of morphological responses to durophagy suggest that this characterization does not hold for our results.

Imperfect convergence is consistent with a model of morphological evolution toward a diet-associated optimum, but substantial constraints prevent species from reaching the adaptive peak. Hansen (1997) describes this scenario as an OU process in which lineages that share a selective regime evolve toward a primary phenotypic optimum but are slowed in their approach by lineage-specific constraints, which lead to variable secondary optima for species. The

Table 2: Correlations between individual trait trajectories and their ancestral states across durophagous transitions

Morphological trait	r	t statistic	P
AM mass	−.15	−.436	.674
Lower jaw closing MA	−.76	−3.330	.010
Mx length	−.39	−1.210	.261
PMx-Et-V area	−.13	−.360	.728
SO length	.69	2.673	.028
Mx tooth length	−.36	−1.081	.311
Mx tooth width	−.35	−1.067	.317
PMx-Et-V tooth length	.26	.769	.464
PMx-Et-V tooth width	.07	.187	.856

Note: P values based on Student's t distribution with 8 degrees of freedom ($n = 10$ trajectories).

OU model's strength of selection parameter (α) dictates the extent to which these secondary optima are spread across trait values, with larger α describing less among-species variation (Hansen 1997). Imperfect convergence in morphospace can therefore be described as an OU process under moderate α, in which the effects of both adaptation and constraint are apparent, and the degree to which convergence is imperfect can be quantified by α or its corresponding phylogenetic half-life ($=\ln(2)/\alpha$).

We find considerable variation among individual morphological traits in the degree to which they converge, a pattern that contributes to imperfect convergence. Some aspects of morphology consistently evolve during dietary transitions, and these traits align evolutionary trajectories through morphospace (fig. 2). But others are more variable, generating a mosaic of evolutionary responses among traits across lineages (fig. 4) and resulting in diverse morphological outcomes (fig. 2). We stress that this pattern need not arise simply because some morphological traits are primarily involved in functions relating to alternative niche dimensions (as shown in Harmon et al. 2005) or are unimportant to the aspect of performance under selection; we document imperfect convergence in a suite of skull traits that are expected to contribute to feeding performance on a single functional prey category. Instead, varying responses among traits are likely the result of some of the same factors that cause lineages to reach varying outcomes under shared selection (see below).

We argue that imperfect convergence is a useful concept in macroevolution because it highlights trait-by-trait variation in the relative importance of selection to a specific adaptive peak versus lineage-specific constraints. In this respect, our results have much in common with previous studies that have identified variation in the degree of convergence across features of the organism (see Langerhans and DeWitt 2004; Harmon et al. 2005; Langerhans et al.

2006; Hendry et al. 2011; Kaeuffer et al. 2011). In particular, Langerhans and DeWitt (2004) advanced a conceptual and statistical framework for partitioning morphological responses into those that are shared versus unique among lineages experiencing a common selection gradient. Like taxa that exhibit both shared and unique responses, imperfect convergence points to significant deterministic effects of selection and lineage-specific constraints. Unlike the shared-versus-unique framework, which focuses on similarity in evolutionary trajectories, the concept of imperfect convergence developed here connects trajectories to the patterns of morphospace occupation that are often used to assess convergence (Stayton 2006; Revell et al. 2007) or to detect adaptive evolution in general (Hansen 1997; Butler and King 2004; Beaulieu et al. 2012). Nevertheless, we expect that both the shared-versus-unique framework and our approach to investigating imperfect convergence will aid in identifying parts of the organism that are most responsive to selection.

Imperfect Convergence in Moray Eels

In moray eels, 10 independent shifts to durophagy from piscivorous ancestors resulted in cranial morphological evolution consistent with enhanced feeding performance on hard prey, but responses varied among traits. Although durophagous transitions involve trajectories through morphospace (PCs 1 and 2) that are more similar in direction than the background trajectories leading to piscivores, durophagous trajectories do exhibit some variation in direction. While seven trajectories point toward decreasing PC 1 and increasing PC 2—the expected direction of change for enhanced durophagous performance—three trajectories are nearly orthogonal, pointing toward decreasing PC 1 but also decreasing PC 2 (figs. 2, 3). Individual trait trajectories shed light on this variation. Three traits—maxilla length, maxillary tooth length, and PMx-Et-V tooth length—decrease with every transition to durophagy, and in the majority of cases, their trajectories are significantly greater than background trajectories in piscivores (fig. 4). These traits—as well as lower jaw MA, which changes as expected in almost every transition—load strongly on PC 1, indicating that this morphological axis describes the combination of traits responding most consistently and dramatically to selection associated with durophagy. Principal component 2, on the other hand, loads strongly with traits exhibiting less consistent responses. In particular, PMx-Et-V area varies most in trajectory direction among cranial features and has the strongest loading on PC 2 (table A3).

The failure of durophagous morays to meet the derived similarity requirement of convergence could also be a consequence of their descent from the same ancestral diet.

Although convergence generally describes evolutionary responses in lineages that differ in starting states (Stayton 2006), durophagous morays are likely all descended from piscivorous ancestors. Capture of large, evasive prey, such as fish and squid, may impose its own strict selective demands (Collar et al. 2009). Indeed, piscivorous morays occupy a relatively restricted region of morphospace, reflecting adaptations for apprehending their prey, such as elongated jaws and large teeth on the upper jaw and PMx-Et-V plate (fig. 2). Similarities among durophagous lineages in morphological starting points likely prevented a reduction in disparity following transitions to durophagy despite its selective demands. Nevertheless, variability in evolutionary trajectories among transitions still requires explanation.

Differences in how readily aspects of the skull respond to durophagy-imposed selection may be related to intrinsic constraints on trait evolution. The three features that always responded to durophagy in the predicted direction are the only ones in which durophagous adaptation involves reductions in trait size. Evidence from other taxa indicates that diminution or loss of structures evolves more rapidly than enhancements or gains (Hecht and Edwards 1977; Fong et al. 1995; Brandley et al. 2008). This phenomenon likely has a molecular basis, as deleterious mutations arise more readily to downregulate gene pathways responsible for the growth of morphological structures (Oakley et al. 2006; Whittall et al. 2006).

Although all nine cranial traits are involved in feeding performance on hard prey, differences among traits in their performance gradients presumably also contribute to the observed variation in evolutionary trajectories. After maxilla length and tooth lengths, the traits responding most consistently to durophagy are lower jaw closing MA and AM muscle mass (fig. 4)—the two cranial features that are directly involved in generating bite force. In fact, the few lineages showing reductions in lower jaw MA (*Gymnothorax buroensis*) or AM mass (*Gymnothorax* group 1 and the lineage including *Echidna leucotaenia* and *Echidna polyzona*, referred to as *Echidna* group 2) experience evolutionary increases in the other bite force-generating trait (i.e., AM mass increases in *G. buroensis* and lower jaw MA increases in *Gymnothorax* group 1 and *Echidna* group 2), suggesting that these lineages may experience no decrement in bite force. These traits may change consistently across durophagous lineages because the strength of the bite is tightly linked to feeding performance on hard prey. In contrast, supraoccipital length and PMx-Et-V area, which vary to a greater extent across transitions (fig. 4), reinforce the skull against large bite forces, and may have less of an effect on feeding performance.

Factors Contributing to Variability among Lineages in Response to Durophagy

Variation among traits in their responses to durophagy is also a consequence of lineage-specific factors that dictate exactly which traits are able to change most. In fact, we found that every independent transition to durophagy exhibits a unique combination of cranial modifications (fig. 4). Below, we consider the evidence for several factors that can contribute to this among-lineage diversity.

Variation in Ancestral Morphology. Transitions to durophagy may involve little morphological change if piscivorous ancestors already possessed traits that perform well at crushing prey. In general, we found that this explanation is inconsistent with our results. Seven of nine traits show relatively weak, nonsignificant correlations between trajectories and their ancestral values, and only lower jaw MA exhibits a strong correlation in the negative direction predicted by this explanation (table 2). We note, however, that variation among ancestors in unmeasured traits could potentially contribute to variable responses among lineages if they are linked to the focal traits by genetic correlations (Lande 1979; Lande and Arnold 1983) or contributions to other shared functions (Walker 2007).

Variation in Selection Imposed by Durophagy. We found little support for the hypothesis that morays specializing on hard prey experienced selection toward a morphological optimum separate from the one for morays that eat a mix of hard-shelled and evasive prey (table 1; table A5). We note, however, that some moray species in our sample were excluded from this analysis because diet data were unavailable, and diet characterizations for some species are uncertain because of limited sample sizes (see table A2). We therefore acknowledge that additional diet information is required before this hypothesis can be thoroughly rejected.

Another reason why durophagy-imposed selection may vary is that morays can use different behaviors to extract prey from their shells. In addition to crushing by biting, moray eels are known to use rotational feeding and knotting to remove pieces of prey (Kondo 1955; Miller 1987, 1989). Both of these behaviors involve use of body movements to apply additional force to remove pieces of prey while the prey is held in the oral jaws. Some morays may utilize alternative behavioral strategies like these to lessen the selection gradient on bite force and the skull morphologies that generate it.

Lineage-Specific Constraints on Evolution. Although they share a diet of hard prey, durophagous morays likely differ in intrinsic properties and environmental backgrounds

that affect the capacity for cranial traits to respond to durophagy-imposed selection. We quantified the overall resistance to adaptation that results from these factors as phylogenetic half-life inferred from OU models (Hansen 1997; Hansen and Orzack 2005). The two-peak (θ_{pisc} and θ_{duro}) OU model providing the best fit to PC 1 inferred a phylogenetic half-life of 8.9 myr, which is longer than the time to the MRPA for two durophagous transitions and between 20% and 83% of the age of the remaining MRPAs (fig. 2). This half-life estimate therefore suggests a significant effect of lineage-specific constraints on adaptation along PC 1, which loads strongly with the suite of traits that most reliably change in association with transitions to durophagy. Phylogenetic half-life was considerably longer for PC 2, reflecting lineage-specific constraints that overwhelm diet-imposed selection on this axis.

Although constraints within lineages likely impeded evolutionary responses to durophagy, our data do not allow us to identify exactly which intrinsic or environmental factors are responsible. The cranial traits examined in our study either directly contribute to (or are correlated with traits that contribute to) other biological functions, like swimming, respiration, and sensing the environment, which may impose competing selection gradients (or differentials [sensu Lande and Arnold 1983]). Moray species may differ in aspects of the environment, like habitat or depth, leading to variation in selective demands on skull functions and in the strength of functional trade-offs. Unfortunately, few data are available to identify relevant environmental differences among moray lineages, and what is known about a few well-studied morays (e.g., *Gymnothorax moringa*, *Gymnothorax vicinus*) suggests that they may be ecological generalists occupying a wide variety of habitats (Young and Winn 2003). In addition, the roles of genetic or developmental factors in constraining evolution of the morphological traits examined in this study are only beginning to be uncovered (Albertson et al. 2005; reviewed in Hulsey et al. 2006).

Many-to-One Mapping of Form to Function. Even when selection on performance is uniform across lineages, independent transitions may reach different morphological endpoints if the relationship between morphology and performance is many-to-one (Alfaro et al. 2004, 2005). Because many features of musculoskeletal anatomy and physiology contribute to the ability to consume hard prey (Westneat 2003), we expect the map of cranial morphology to durophagous performance to be many-to-one. However, without either a direct measure of feeding performance or a quantitative biomechanical model capable of estimating performance from our morphological data, we are unable to determine whether the morphological diversity we observe among durophagous species corresponds to similar or variable feeding performance. We found evidence, however, that the two traits in our data set directly involved in generating bite force—lower jaw MA and AM muscle mass—exhibit different combinations of changes across durophagous transitions: *Gymnomuraena zebra* and *Echidna* group 1 show exceptional evolutionary trajectories for both traits, four lineages (*Scuticaria tigrina*, *Pseudoechidna brummeri*, *Gymnothorax pictus*, and *Gymnothorax prasinus*) show increases in both traits, but at least one trait is unexceptional compared to piscivores, and three lineages (*Echidna* group 2, *Gymnothorax* group 1, and *G. buroensis*) show decreases in one trait but increases in the other (fig. 4). This variation in evolutionary trajectories suggests that multiple combinations of traits may be able to enhance bite force capacity during durophagous transitions, though without direct measures of bite force, we are unable to confirm this inference.

Conclusions

Multiple independent transitions to durophagy in moray eels resulted in repeated evolution toward a novel region of morphospace, reflecting shared phenotypic responses to the demands of consuming hard prey. However, the elevated morphological disparity of durophagous species is likely a consequence of constraints on the capacity of some traits and some lineages to adapt to the derived diet state. Our results therefore suggest, somewhat paradoxically, that constraints on adaptation can actually enhance diversification within clades. The morphological optimum associated with the derived diet pulls species away from the ancestral diet-associated optimum, but if all traits and lineages were free to adapt, species in the derived state would be at the optimum (Hansen 1997)—the pattern expected under convergence. By preventing some species from reaching the derived morphological optimum, lineage-specific constraints generate a pattern of imperfect convergence in which species reach suboptima spread more broadly across morphospace (Hansen 1997). Alternatively, variable constraints or chance events may lead species to different morphological endpoints along an adaptive ridge if the morphology to performance relationship is many-to-one (Alfaro et al. 2004, 2005). Although identification of convergent patterns has been important for understanding adaptation and determinism in evolution, we propose that a shift toward recognizing imperfect convergence will shed light on mechanisms shaping broader patterns of diversity. The combined influence of functional transitions plus trait- and lineage-specific constraints on adaptation is likely to be a major engine of morphological diversification.

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The chestnut moray eel (*Gymnothorax castaneus*) emerging from a crevice near Cocos Island, Costa Rica. Photo credit: Jonathan T. Redwine.