

EVOLUTIONARY DYNAMICS OF COMPLEX BIOMECHANICAL SYSTEMS: AN EXAMPLE USING THE FOUR-BAR MECHANISM

MICHAEL E. ALFARO,¹ DANIEL I. BOLNICK,² AND PETER C. WAINWRIGHT

Section of Evolution and Ecology, One Shields Avenue, University of California, Davis, California 95616

Abstract.—Like many phenotypic traits, biomechanical systems are defined by both an underlying morphology and an emergent functional property. The relationship between these levels may have a profound impact on how selection for functional performance is translated into morphological evolution. In particular, complex mechanical systems are likely to be highly redundant, because many alternative morphologies yield equivalent functions. We suggest that this redundancy weakens the relationship between morphological and functional diversity, and we illustrate this effect using an evolutionary model of the four-bar lever system of labrid fishes. Our results demonstrate that, when traits are complex, the morphological diversity of a clade may only weakly predict its mechanical diversity. Furthermore, parallel or convergent selection on function does not necessarily produce convergence in morphology. Empirical observations suggest that this weak form-function relationship has contributed to the morphological diversity of labrid fishes, as functionally equivalent species may nevertheless possess morphologically distinct jaws. We suggest that partial decoupling of morphology and mechanics due to redundancy is a major factor in morphological diversification.

Key words.—Biomechanics, character evolution, convergence, disparity, four-bar, Labridae, natural selection.

Received July 8, 2003. Accepted November 7, 2003.

A mechanical trait in an organism possesses at least two, hierarchically related levels of biological interest: an underlying combination of parts, and an emergent property of those parts upon which selection acts. In the simplest cases, trait mechanics or function will simply and directly reflect the underlying morphology. Such examples might include tooth shape or muscle cross-sectional area. In more complex cases, as in most behavioral and functional characters, trait function will be an emergent property of a combination of possibly many morphological parts. For example, a number of teleosts feed on snails by crushing shells (Hernandez and Motta 1997; Ralston and Wainwright 1997; Grubich 2000). These fishes have all evolved the ability to generate powerful bite forces with their jaws, although the exact way in which this function is accomplished varies substantially across species. Most strikingly, some taxa rely on the oral jaws to generate high crushing forces (Hernandez and Motta 1997; Ralston and Wainwright 1997), whereas others use robust pharyngeal jaws (Lauder 1983; Liem and Kaufman 1984; Wainwright 1987). Even within the pharyngeal jaw crushers, there exists high variation across species in the pattern of muscular recruitment used to accomplish the crushing bite and in details of pharyngeal musculoskeletal anatomy (Liem and Kaufman 1984; Wainwright 1987; Grubich 2000).

Although it is not clear which (if any) of these morphological differences are due to other differences in the ecology of these species, the results of selection experiments suggest that selection at the level of function may sometimes produce a diversity of solutions at underlying levels. For example, fruitfly lines under identical selection regimes for alcohol resistance show increased rather than decreased genotypic variation (Hoffman and Cohan 1987; Cohan and Hoffman

1989), and mice selected to be good runners on wheels evolve a range of physiological responses (Garland 2003).

The dynamic between trait form and function holds important implications for the study of trait evolution. If there exists a redundant or many-to-one mapping of trait form to trait function, then unique morphologies do not imply unique functions. In such a system, it is theoretically possible for clades to be both morphologically diverse yet functionally depauperate (or vice versa), complicating studies of adaptation and morphological evolution. Convergence is generally recognized as the strongest evidence for adaptation in comparative studies (Pagel 1994; Larson and Losos 1996). However, if functional convergence is accompanied by a high degree of morphological diversity, adaptive evolution may go unrecognized unless all functionally equivalent types are identified. Similarly, redundant mapping means that morphological evolution may be functionally neutral (morphological drift), obscuring the evolutionary significance of morphological change in traits. Molecular studies of ribosomal RNA folding constitute some of the best examples of the evolutionary consequences of redundant systems (Schuster et al. 1994; Fontana and Schuster 1998; Schuster and Fontana 1999; Stadler et al. 2001). The topic of trait diversity in response to uniform selection has been little explored outside of studies in model systems such as bacteria (e.g., Lenski and Travisano 1994; Korona 1996; Travisano and Lenski 1996) and *Drosophila* (e.g., Hoffman and Cohan 1987; Cohan and Hoffman 1989). Here we explore the evolutionary dynamics of a complex functional trait using biomechanical and evolutionary models of the jaws in labrid fishes.

Levers are common mechanisms that are used to transmit force and motion. The lower jaw of a fish is an example of a simple biological lever system and possesses two lengths: an input lever, where force is applied, and an output lever, which transmits the force (Fig. 1A). Kinematic transmission (KT), which expresses the amount of movement expressed at the tip of the output lever per unit of input rotation, is a simple function of the ratio these lengths (Barel 1977). KT

¹ Present address: Section of Ecology, Behavior, and Evolution, Division of Biological Sciences, University of California, San Diego, La Jolla, California 92093-0116; E-mail: malfaro@ucsd.edu.

² Present address: Section of Integrative Biology, School of Biological Sciences, University of Texas, 1 University Station CO930, Austin, Texas 78712-0253.

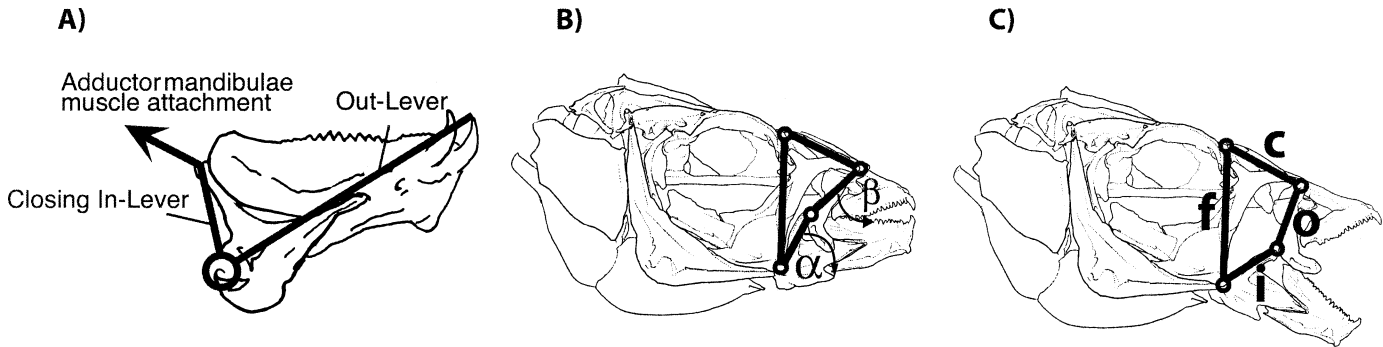


FIG. 1. Simple and complex levers in labrid fishes. (A) Morphology in simple levers, like the lower jaw of a fish, determines emergent functional properties in a straightforward way. Here kinematic transmission (KT) is equal to the ratio of the closing out-lever to the in-lever. (B) Four-bar mechanism in closed position. Four-bars are complex levers that are found in the jaws of many fishes that function to protrude the jaws during prey capture. Mechanical properties of the four-bar like maxillary KT (the ratio of output rotation, β , to input rotation, α), are complex emergent functions of the underlying morphology. The effect that a change in length of one linkage element will have on the functional property of the mechanism can only be determined by knowing the geometry of the other elements. (C) Four-bar in open position: f, fixed link; i, input link; c, coupler link; o, output link.

is a size-independent mechanical property because it depends only on the shape (ratio of output and input lengths) of the jaws and is unaffected by the absolute length of the elements themselves. As a result, the map between jaw shape (morphology) and jaw mechanics is simple in that it is not redundant (i.e., one jaw shape maps to a unique value of KT). Thus, mechanically similar species will have similarly shaped lower jaws, and a clade in which diverse morphologies are present will also be a clade that is mechanically rich (Bouton et al. 2002; Lovette et al. 2002).

Four-bar linkages are commonly encountered in mechanical engineering but also characterize skeletal arrangements in diverse animal systems (Muller 1996) including fish jaws (Westneat and Wainwright 1989; Westneat 1990, 1991, 1994; Muller 1996; Hulsey and Wainwright 2002; Fig. 1B, C). Four-bars are complex levers in which four linked elements (the fixed link, input link, coupler link, and output link) function to transmit force and motion. The rigid links connect at four joints to form a movable, closed, coplanar rhombhedron with one degree of freedom (meaning that for any given angle between two links, all remaining angles of the mechanism are determined). Thus, rotation applied to the input link results in movement of the coupler and output links in a deterministic way.

One of the primary functions of the four-bar system in labrids is hypothesized to be the protrusion of the premaxilla via rotation of the maxilla (Westneat and Wainwright 1989; Westneat 1990, 1994). The degree and rate of premaxillary protrusion in turn are thought to be important determinants of foraging ecology (Westneat 1994, 1995; Norton 1995; Wainwright and Bellwood 2002).

The kinematic transmission coefficient (KT) for maxillary rotation may be calculated for the four-bar, and like the simple lever example above, relates the amount of maxillary rotation produced by a given amount of lower jaw rotation. Just as in the simple lever system already discussed, KT is a function of the shape of the four-bar (the relative length of the links to one another) but is independent of absolute size. There are two important differences between simple levers and four-bars with respect to mechanics. First, four-bar mor-

phology maps nonlinearly to KT (Muller 1996). As a result, small changes in four-bar morphology will not necessarily produce proportionate changes in KT (Hulsey and Wainwright 2002). Second, four-bar shape maps redundantly (Stadler et al. 2001) to KT, that is, multiple combinations of four-bar lengths can combine to produce the same mechanical property (Hulsey and Wainwright 2002). Thus, whereas a simple lower jaw lever shows a linear, one-to-one relationship between morphology and KT, a many-to-one relationship characterizes four-bar mechanisms.

These properties of the four-bar mechanism may influence the evolution of mechanical and morphological diversity in at least two important ways. First, because evolutionary change in morphology will not necessarily produce change in trait mechanics, morphological measures of diversity in a clade may not perfectly correspond to mechanical or ecological measures of diversity (Koehl 1996). Although morphology is often used as a proxy for function in comparative ecological studies (Gatz 1979; Wiens and Rotenberry 1981; Losos 1990; Winemiller 1991; Warheit et al. 1999), the nonlinear and redundant nature of form-function mapping in complex traits suggests that the true correlation between the measures may be poor. Second, because multiple morphologies can produce equivalent functions, the evolutionary response of trait morphology to selection on mechanics may be difficult to predict.

The labrid four-bar is an ideal system for the study of the evolutionary dynamics of complex traits. The mechanism is moderately complex, and the relationship between its morphology and mechanics is not obvious or intuitive. Despite this morphological complexity, it is easy to parameterize (Hulsey and Wainwright 2002), and the calculation of its mechanics is straightforward given starting and ending geometries. Finally, the trait has demonstrated functional and ecological relevance to the organism (Westneat 1990, 1995). Thus, it is one of a few morphological systems in which it is possible to model the effects of evolutionary change in a property at one level on the elements of another level. Despite the large number of studies relating morphological diversity to functional or ecological diversity, there currently exists

no theoretically derived expectation for this relationship. Thus, study of this relationship in the four-bar in simulation may provide novel perspective on a question of general interest in evolutionary biology.

Here we examine the relationship between morphology and mechanics using both phylogenetic and genetically explicit simulations of four-bar evolution. The objective of our simulations is to describe expected patterns of behavior of a complex trait under simple models of character evolution and to see if these models are consistent with some assumptions that are commonly made in comparative studies. Specifically, we tested the hypothesis that morphology predicts mechanical diversity by simulating the evolution of the four-bar links across a phylogeny and calculating mechanical and morphological variance. If morphology is a strong predictor of mechanics, then the correlation between morphological and mechanical diversity should be high.

We also used a genetically explicit model to test whether, given three different starting four-bar morphologies, natural selection for convergent mechanics will produce morphological similarity in the ending populations. The nature of the map between morphology and mechanics is expected to determine the outcome of this experiment. If the system possesses only a small degree of redundancy, then selection on mechanics should produce four-bars that are morphologically similar across all three starting populations. If the system is highly redundant, then mechanically similar ending populations are expected to be morphologically dissimilar.

Finally, we measured jaw morphology in living labrids to explore the degree of morphological diversity associated with mechanical equivalence in living fishes. Our analysis is ahistorical due to the absence of a higher-level phylogeny for the labrids. Nevertheless, we expect that if mechanical and morphological diversity are strongly correlated, we will find limited morphological diversity within mechanically equivalent groups of species.

Some caveats apply. We focus on the four-bar and KT, a single emergent property of the mechanism. This represents a greatly simplified view of the function of the four-bar in a living fish. The bony elements comprising the four-bar are central features of the skull and are almost certainly involved in roles in addition to jaw protrusion. It is also possible that there are important secondary functions of the four-bar mechanism itself (although none have yet been suggested) or that other mechanical properties are also relevant. Although our model may be a simplification, we argue that it is biologically justified by our current understanding of the four-bar system. Furthermore, our approach of focusing on a single mechanical property reflects the practice of many morphologists and ecologists, who typically consider a single—or at most a small number of—functional axes in the study of trait function.

MATERIALS AND METHODS

Four-Bar Mechanics

Like crowbars and other simple lever systems, the four-bar mechanism functions to transmit force and motion. In labrid fishes, muscular contraction imparts a rotation on the lower jaw (input link) relative to the fixed link (Westneat 1990; Fig. 1). This rotation is transmitted to the jaws via the

maxilla (output link) and results in protrusion of the premaxilla, which is thought to be an important component of feeding performance (Westneat 1994, 1995; Norton 1995; Wainwright and Bellwood 2002). We measured the angular rotation of the maxillary link for 30° of lower jaw rotation and used this ratio, termed the kinematic transmission coefficient (KT), as one measure of the mechanics of this system (Hulsey and Wainwright 2002). KT represents an emergent functional property of the four-bar morphology.

Phylogenetic Evolution of the Four-Bar Linkages

Because four-bar morphology is known to have a many-to-one mapping onto KT (Hulsey and Wainwright 2001), it is possible that for this system morphological diversity is partially or totally decoupled from mechanical diversity. To test this, we simulated the macroevolution of the four-bar system in wrasses, in which we repeatedly allowed morphological traits to evolve on a phylogeny. We then tested whether the morphological variance among the taxa at the tips of the phylogeny was a good predictor of the mechanical variance. For each replicate of the simulation (written in JAVA), the program generated a 500-taxon tree, created the ancestral state of the four-bar mechanism, evolved the links from their starting condition across the tree, and calculated variance in link morphology and KT across simulated tip species.

Trees were generated under a stochastic Yule birth process conditioned on the approximate size (500 taxa) and age (65 million years) of the labrids. The resulting trees thus represented a subsample of all ordered branching histories (e.g., Martins 1996; Housworth and Martins 2001) constrained to have evolved 500 terminals in exactly 65 million years. By evolving our characters on trees from this stable age distribution (Ross 1983), we ensured that the recovered relationship between morphological and mechanical diversity was not confounded by underlying differences in fundamental tree shape (Bininda-Emonds et al. 2001).

Currently, higher-level relationships within the labrids are not understood, making the inference of the ancestral condition of the four-bar problematic. We choose to vary the ancestral state of the starting four-bar to reflect our uncertainty about the states of the links in early labrids and to generalize the results of our simulations to all biologically realistic four-bar geometries rather than a small number of specifically chosen values. We fitted normal distributions to link measures taken from 119 labrid species in a previous study (Wainwright et al. 2004) and randomly drew from them to create starting four-bars morphologies for each simulation replicate. Taxon sampling within the empirical dataset is representative of the ecological and morphological diversity of the family and also covers members from each major tribe. Given this we do not expect that more complete taxonomic sampling would substantially alter the distributions, we used to generate four-bar starting conditions. Thus, we believe that the results from our simulation would not substantially change with more inclusive sampling of the labrids.

We chose a Brownian model of character evolution (Felsenstein 1985) because we were interested in describing the theoretical (null) relationship between four-bar mechanics and morphology in the absence of selection on either prop-

erty, and Brownian motion best describes continuous characters evolving via drift (Felsenstein 1988; Hansen and Martins 1996). Trait evolution was constrained so that the summed lengths of any three links always exceeded the length of the remaining link. This preserved the functional integrity of the four-bar mechanism for every node. Biologically realistic boundaries were also placed on the raw lengths of the links and on the relative lengths of the links to one another. These boundaries corresponded to the minimum and maximum observed in the morphometric dataset for each link. During simulations, proposed changes to element length were redrawn until the new state fell within the boundary conditions.

The mechanics of the four-bar system are scale independent. To remove the effects of size on variance calculations, the program expressed the lengths of the input, coupler, and output links as a fraction of the fixed link. The program also calculated the maxillary KT for each of the 500 terminal taxa. We then measured morphological diversity as the summed variance in length of the three scale-independent links and mechanical diversity as the variance in KT. We replicated the simulation 1000 times. We evaluated the goodness of morphology as a predictor for mechanical diversity by regressing KT variance on link variance. When boundaries are placed on trait evolution, as they were in our simulations, overall variation in the trait is reduced relative to a truly Brownian model (Diaz-Uriarte and Garland 1996). Sensitivity analyses across a 10% change in link length, however, revealed that the exact choice of boundary values had a negligible effect on the general pattern of relationship between morphological and KT variance.

Our simulation parameters were chosen to explore the relationship between morphology and KT across a wrasse-bounded morphospace. Because most four-bars have the potential for redundant mapping of morphology to single mechanical properties, we expect that our results will be generally applicable to other biological four-bar mechanisms (Muller 1996). Behavior of morphology and mechanics in this simple system may also have heuristic value in shaping our expectation for the relationship between form and function for complex traits in general.

Explicit Genetic Model of Selection

As noted in the introduction, one possible consequence of the many-to-one mapping of morphology on KT is that natural selection on a functional property may have unpredictable effects on the underlying morphology. In particular, parallel or convergent functional evolution may not result in morphological convergence. To test this, we used an individual-based computer simulation (written in LISP), in which stabilizing selection is applied to the emergent function (KT) of a set of heritable morphological traits. We can thus observe how morphology changes as the functional trait: (1) evolves in parallel as identical replicate starting populations evolve directionally to the same functional optimum and (2) converges as starting populations with disparate functions evolve to the same optimum.

Each simulation began with a population of 500 individuals. This small population size might induce some genetic

drift, but given the strong selection and short duration of the simulations (500 generations) its effect on the end results is negligible. To model the genetic basis of the four morphological traits, we used an additive multilocus model with uniform allele effects, approximately following Dieckmann and Doebeli (1999) and Bolnick and Doebeli (2003). Every individual was assigned a haploid genotype that determined its four morphological traits (fixed link, connecting link, input link, and output link). The genotype consisted of 50 loci for each of the four traits. Any given locus could have an allele of value 0 or 1. The summed value of all 50 loci in an individual yielded the length of the corresponding morphological trait, which thus ranged from zero to 50. Our choice of 50 loci reflects a compromise between conflicting demands: More loci better approximate a continuous distribution, but greatly inflate computing demands. The outcome of the simulations appears to be robust to this choice (based on limited reruns with 25 and 100 loci). We assume that there is no phenotypic plasticity. Because our goal is to investigate the relationship between morphology and function, this additional source of variance could obscure the core point of the simulations.

Without restrictions on the initial genetic variance of each trait and without covariance between traits, simulated populations would have unrealistically large intraspecific variance in KT (exceeding the variance of the entire family Labridae). This was avoided in two ways. First, all but two loci were fixed within any given population, so that initially the maximum range of any given link length was two units rather than 50. For example, to set a starting mean link length of 40, we generated a population that is fixed for the 1 allele at 39 loci, fixed for the 0 allele at nine loci. For the two remaining loci, individuals were randomly assigned 0 or 1 alleles with probabilities that were chosen to achieve a particular mean—in this case 0.5 for both 0 and 1 alleles. Second, we built in a correlation among the four traits. For any given individual, the two variable loci were randomly assigned alleles only for the first trait (the fixed link). The variable loci for the other three traits were given allele values identical to those assigned to the fixed link. On rare occasions ($p = 0.01$), the allele value was reversed. These settings yielded more reasonable intraspecific variances, which were maintained during the 500 generations of simulation by also incorporating a correlation among the traits during recombination (see below).

After assigning each individual a genotype and phenotype, the population was subjected to stabilizing natural selection on KT, which was calculated using the relative lengths of the three mobile links, scaled to the length of the fixed link, and assuming an input angle of 30° (Hulsey and Wainwright 2002). Each individual had a probability of surviving based on the difference between its biomechanical trait (KT_i) and the optimal biomechanical value ($KT_{optimum}$). Specifically,

$$P(\text{survival}) = \exp\left[-\frac{1}{2} \frac{(KT_i - KT_{optimum})^2}{\sigma_w^2}\right], \quad (1)$$

where σ_w is a measure of the intensity of stabilizing selection.

Survivors were randomly divided into pairs, and each pair produced a number of offspring drawn randomly from a Poisson distribution with mean $n = 1 + r(1 - N/K)$, where r is

the natural rate of increase, N is the number of parents surviving selection, and K is the carrying capacity. For all simulations, r was set equal to one and K equal to 1000. An offspring was assigned one allele per locus, drawn randomly from either parent. To simulate genetic correlation between traits, there was a 0.99 probability that if the fixed link allele for the i th locus was drawn from a given parent, the alleles for the i th loci for the other three traits would also be drawn from that parent. This large correlation among traits was necessary to maintain a realistically low within-population variance in KT , otherwise recombination would quickly inflate the variance to unrealistic magnitudes. In addition, this correlation reflects body size: the tendency for all links to get larger or smaller in concert without changes in shape. For each allele passed on to an offspring there was a probability $m = 0.0001$ that the allele reverses its value (representing mutation). Selection and mating were iterated for 500 generations per simulation, long enough to ensure that all populations reached a stable mean KT , without unnecessary additional time for drift to cause neutral morphological divergence.

We chose three biomechanically divergent morphologies as alternative starting points for simulations. These morphologies were chosen from actual jaw shapes observed in individual specimens of three wrasse species (*Anampses twistii*, maxillary $KT = 0.68$; *Pseudolabrus guenteri*, $KT = 0.98$; and *Cirrhilabrus laboutei*, $KT = 1.57$). In separate simulations, each of these starting morphologies was subjected to stabilizing selection with varying optima ($KT = 0.5, 1.0$, and 1.5). For each combination of starting morphology and optimum KT , we ran 100 replicate simulations, recording the mean four-bar shape and KT at the end of 500 generations. For brevity, we report the results of the simulations with $KT_{optimum} = 1.0$, as qualitatively similar phenomena were observed for all three optima.

For comparison, we repeated the selection simulations using a simpler biomechanical trait, lower jaw KT (Fig. 1A). This value is the ratio of the lengths of the output arm to the input arm. We generated populations in which individuals were characterized by two morphological variables rather than four. Selection then acted on the difference between an individual's lever ratio and the optimal lever ratio. Starting and optimum ratios were identical to starting and optimum values of four-bar KT , above.

Morphometrics

To determine if morphological diversity was strongly correlated with mechanical diversity in living labrids, we explored the variance in four-bar morphology of species with mechanically similar jaws. Linkage morphology and maxillary KT s were measured for 119 Great Barrier Reef labrids using previously described methods (Wainwright et al. 2004). These labrids occupied a diversity of trophic niches as well; piscivores, molluscivores, planktivores, and crustacivores were all represented within our sample (Westneat 1999). We calculated the lengths of the four-bar elements and expressed them as a fraction of the fixed length to remove the effects of size. Ninety-five percent of the sampled species had KT s of between 0.50 and 1.29. To quantify the amount of diversity

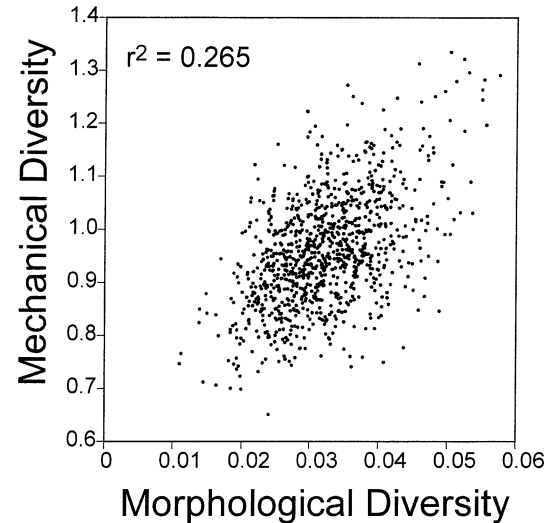


Fig. 2. Morphological and mechanical diversity in simulated phylogenies. Each point represents the outcome of one replicate of simulated oral jaw evolution in wrasses. For each replicate we first randomly generated a 500-taxon tree. Then we generated ancestral states for each of the four elements of the four-bar linkage system and evolved those links over the tree using a Brownian model of character change. Morphological diversity, calculated as the summed variance in each of the links, and mechanical diversity, calculated as the variance in maxillary kinematic transmission across the tree were positively and weakly correlated ($r^2 = 0.265$).

in morphology possessed by mechanically similar wrasses we divided this range of KT s into bins one-tenth of a KT wide. We then calculated the morphological variance present in each bin and compared that to the total variance across all species. We also expressed the bin sample range in length for each of the four links as a fraction of the range in length across all species as an indicator of four-bar diversity.

RESULTS

Simulated evolution of the four-bar under a Brownian model of character change revealed that within-clade mechanics scales positively but weakly with morphology ($r^2 = 0.265$, Fig. 2). This general result was not sensitive to the ancestral state of the four-bar across biologically realistic lengths and also obtained when tree topology was held fixed (results not shown). This contrasts strongly with simulations of a simple lever (not shown), where morphology perfectly predicted mechanical diversity ($r^2 = 1.0$). Simple models of character change predict that morphological diversity poorly explains mechanical diversity in complex traits.

In our simulations using a genetically explicit model to apply selection to mechanics, all populations evolved a KT of approximately 1.0 (Fig. 3A–C). Apparent morphological evolution could be a poor guide to the amount of resultant mechanical change. For example, in Figure 3B, the mechanics of the starting population lies close to the selected optimum. However, the four-bar appears to have undergone greater morphological evolution to traverse this short mechanical distance than the starting four-bar in Figure 3A, where the starting population is mechanically distant from the target.

Replications of the simulations from the same average

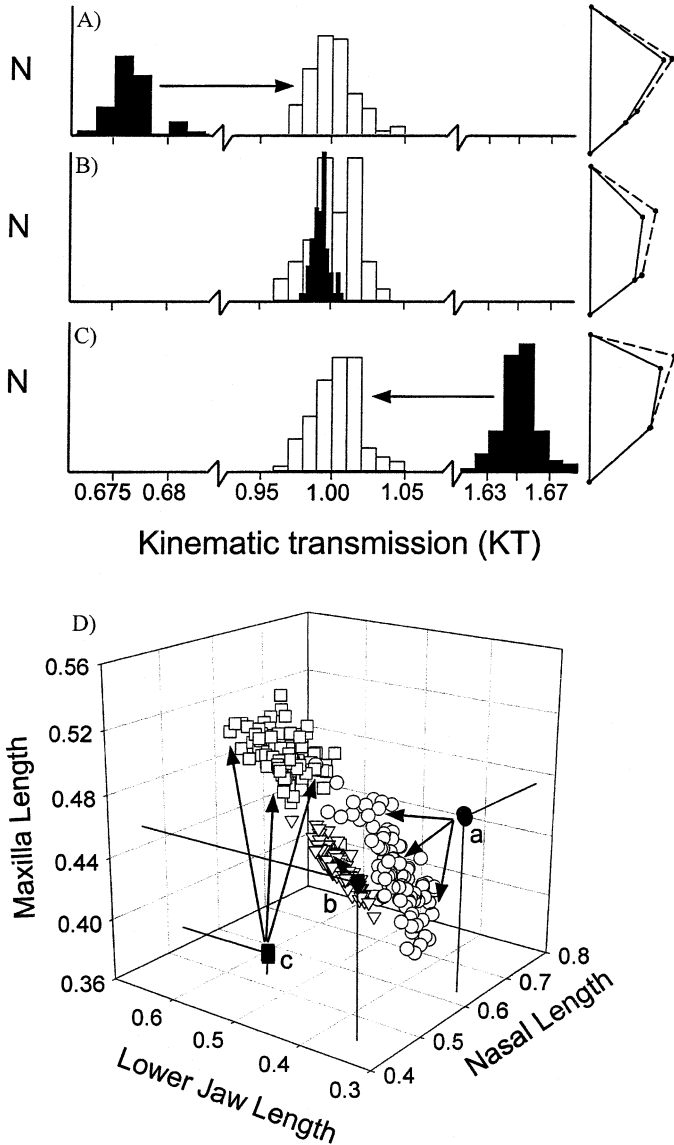


FIG. 3. Simulated populations with different biomechanical properties (black histogram bars in [A] maxillary $KT = 0.67$ [B] $KT = 0.98$, [C] $KT = 1.56$) were subjected to stabilizing natural selection with a fitness optimum of $KT = 1.0$. At the end of 500 generations, populations from all three initial conditions had evolved similar biomechanical traits (white histogram bars, A–C). Starting (solid lines) and ending four-bar morphologies (dotted lines) are drawn to illustrate the shape differences among groups before and after convergent selection. Convergent mechanical evolution did not lead to morphological convergence. The mean jaw shape of each simulated population is indicated by a point in three-dimensional morphospace (D), in which the axes are the relative lengths of the three movable four bar links. These axes are scaled to the length of the fixed link to remove the effect of body size. Filled symbols indicate the morphology of populations prior to natural selection, and white symbols represent population morphologies following selection (circles $KT = 0.67$; triangles $KT = 0.98$; squares $KT = 1.56$). Arrows link initial phenotypes to the range of resulting morphologies. All postselection populations lie approximately on a curvilinear surface corresponding to the $KT = 1.0$ isocurve through morphospace. $KT_{optimum} = 1.0$, $r = 5$, $K = 500$, $\sigma_w = 0.5$.

TABLE 1. Morphological diversity across mechanical range in labrids. The range of kinematic transmissions (KTs) occupied by 95% of the species in the morphological dataset was divided into eight categories one-tenth of a KT wide. The number of species present in each category, the morphological variance of those species expressed as a proportion of the total morphological variance (% variance), and the proportion of the total range in length for each link spanned by those species (% nasal, % max, and % jaw), is shown.

KT	No. species	% variance	% nasal	% max	% jaw
0.50–0.59	11	86	50	45	30
0.60–0.69	20	94	93	53	31
0.70–0.79	25	62	60	44	42
0.80–0.89	28	45	58	27	33
0.90–0.99	14	54	55	37	27
1.00–1.09	4	95	43	18	19
1.10–1.19	8	72	31	34	47
1.20–1.29	4	71	19	42	20

starting location revealed that starting condition and mechanical selection could not be used to exactly predict the ending condition of the four-bar. Instead, a range of morphological solutions could be achieved in response to parallel selection on mechanical property. Populations with different starting mechanics evolved convergent mechanical solutions but did not converge in morphology. Instead, initially divergent populations evolved toward distinct regions of morphospace, coming to occupy different areas of the KT isoplane (Fig. 3D). This implies that starting location placed constraints on morphological evolution.

Empirical measurements of labrids revealed relatively high morphological diversity of species within a restricted mechanical subspace (Table 1). For three KT categories, more than 85% of the total across-species morphological variance was contained within 25% of the species or less. Nasal length was usually the most variable of the three links for a given category, and jaw length was usually the least. This pattern was consistent with the phylogenetic and genetically explicit models of four-bar evolution, which predicted a weak relationship between mechanical and morphological diversity.

DISCUSSION

Under simple models of character evolution, four-bar mechanical diversity does not accumulate at the same rate as morphological diversity within a clade. The strength of correlation between mechanics and morphology in jaw levers appears to be related to the number of parts making up the trait. In our simulations, as the number of parts making up a trait went from two to four, the number of morphological pathways to identical mechanics also increased, with the result that morphological diversity went from a perfect predictor of mechanical diversity to one that was rather poor. We predict that as the number of interacting components increases, the number of morphological solutions to any particular functional problem should also increase. If this is so, then a major effect of increasing trait complexity should be the weakening of the correlation between morphological and functional diversity.

Many traits of real biological interest are far more complex than the relatively simple mechanical system examined in

our study. For example, suction feeding performance in fishes is dependent on the mechanics of a separate four-bar system (Muller 1987; Westneat 1990) plus the geometry of several muscles and their physiological properties (Van Leeuwen and Muller 1984; Muller 1987; de Visser and Barel 1996; Ferry-Graham and Lauder 2001). It is entirely feasible that high-performance suction feeding might be accomplished through several alternative permutations of all of the parts within this system. Given the weak correlation in morphology and mechanics in the four-bar, we suggest that measures of a clade's morphological and ecological breadth may be weakly correlated. Workers assessing ecological or functional diversity should investigate the correlation of morphology and ecology before embarking on large-scale comparative studies. Non-linearity (Koehl 1996) and redundancy both undermine the intuitive notion that morphological diversity reflects functional or ecological diversity (Gatz 1979; Wiens and Rotenberry 1981; Losos 1990; Winemiller 1991) and may seriously weaken the value of statistical tests of ecology that rely on measures of morphology (Ricklefs and Miles 1994; Warheit et al. 1999).

The relative importance of contingency and convergence in shaping patterns of organismal diversity has been the subject of much debate (Gould 1985; Travisano et al. 1995; Foote 1998; Losos et al. 1998). Our genetically explicit model reveals that the evolutionary interplay of form and function in complex traits can produce both of these patterns. When starting populations are morphologically identical and selection acts on an emergent property of morphology, a replaying of the evolutionary tape (simulation replicates in the case of our study) does not produce identical morphological solutions (Gould 1989; Fig. 3D). When starting populations are mechanically and morphologically distinct, mechanical convergence can occur without morphological convergence. The effect of history (starting point) appears to constrain the range of possible morphological solutions that are reached (Fig. 3D). Simpler biomechanical traits, such as the lower jaw lever, cannot produce these patterns because morphological evolution will exactly track mechanical evolution. Thus, the effect of increasing mechanism complexity on the morphology-mechanics relationship may represent a major and understudied aspect of organismal evolution.

Complex mechanisms might also play an important role in promoting morphological diversification through their capacity to evolve shapes that accommodate secondary functions without compromising primary function. For example, the mandible in wrasses is an important component of maximum gape, which has been shown to be an important determinant of feeding performance (Werner 1974; Huskey and Turingan 2001). The mandible is also incorporated in the four-bar linkage. Functional morphologists traditionally would examine this system to determine if there has been an evolutionary trade-off between these two functions. However, it is theoretically possible for the four-bar to achieve a shape that would both possess any specific maxillary KT value and simultaneously allow either a large or small gape. Thus, complex mechanical traits may be able to evolve win-win conformations rather than morphological compromises in response to selection on a particular morphological unit for multiple functions. This capacity could permit organisms

to diversify morphologically and ecologically while meeting functional constraints. Labrids appear to have exploited the nonlinear relationship between morphology and function in their oral jaws, and this may partially account for their spectacular trophic diversity (Wainwright et al. 2004).

More broadly, the four-bar presents the opportunity to study the influence of the map of morphology to function on the evolution of diversity. For example, does an understanding of the nonlinear relationship between morphology and mechanics in the four-bar system help explain differential patterns of mechanical diversity across clades of labrids? The general question has received scant attention from organismal biologists, in part because of the difficulty of modeling complex traits. However, at the molecular level, the genotype-phenotype map has been suggested to strongly affect the evolution of novel structure (Fontana and Schuster 1998; Schuster and Fontana 1999; Stadler et al. 2001). Our simulations suggest the phenotype-function map may, at least theoretically, influence the response of morphology to selection on mechanics in a similar way.

If contours of equivalent mechanics are curved within the wrasse-bounded morphospace as the distribution of ending populations in Figure 3D suggests, then the effect of a unit change to one link on mechanics will be highly dependent on the current position of the four-bar in morphospace. As an example, two different four-bars may both have a KT of 1.0. However, because of their position in morphospace and the slope of the mechanical isocurves, a mutation that changes the length of the nasal could theoretically have a negligible effect on the KT of one four-bar and a large effect on the other. If both four-bars were under selection for a novel KT, selection might act much more quickly on the populations with the second four-bar shape than on those with the first. By extension of the argument, it is possible that the morphological and mechanical diversity of an entire clade could be affected by the position in morphospace of the ancestor. Clearly, additional theoretical and empirical study is required to evaluate such scenarios. However, we are greatly excited by the potential of this system to provide insight into the tempo and mode of morphological and functional evolution.

ACKNOWLEDGMENTS

We acknowledge D. Hulsey and M. Turelli for helpful comments on many phases of this project. M. Westneat provided valuable discussion of four-bar function and evolution in labrids. Comments from J. Weins, D. Irschick, and an anonymous reviewer greatly improved the manuscript. We thank J. Endler, B. Spitzer, and C. Nunn for helpful comments on earlier versions of the manuscript. Funds were provided by National Science Foundation grant IBN 0076436 to PCW.

LITERATURE CITED

- Barel, C. D. N. 1977. Kinematischer Transmissionskoeffizient und Vierstangensystem als Funktionsparameter und Formmodell für Mandibulare Depressionsapparate bei Teleostiern. *Ann. Anat.* 142:21–31.
- Bininda-Emonds, O. R. P., S. G. Brady, J. Kim, and M. J. Sanderson. 2001. Scaling of accuracy in extremely large phylogenetic trees. *Pac. Symp. Biocomput.* 6:547–558.
- Bolnick, D. I., and M. Doebeli. 2003. Sexual dimorphism and adap-

- tive speciation: two sides of the same ecological coin. *Evolution* 57:2433–2449.
- Bouton, N., J. De Visser, and J. Barel. 2002. Correlating head shape with ecological variables in rock-dwelling haplochromines (Teleostei: Cichlidae) from Lake Victoria. *Biol. J. Linn. Soc.* 76: 39–48.
- Cohan, F. M., and A. A. Hoffman. 1989. Uniform selection as a diversifying force in evolution: evidence from *Drosophila*. *Am. Nat.* 134:613–637.
- de Visser, J., and C. D. N. Barel. 1996. Architectonic constraints on the hyoid's optimal starting position for suction feeding of fish. *J. Morphol.* 228:1–18.
- Diaz-Uriarte, R., and T. J. Garland. 1996. Testing hypotheses of correlated evolution using phylogenetically independent contrasts: sensitivity to deviations from Brownian motion. *Syst. Biol.* 45:27–47.
- Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. *Nature* 400:354–357.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* 125:1–15.
- . 1988. Phylogenies and quantitative characters. *Annu. Rev. Ecol. Syst.* 19:445–471.
- Ferry-Graham, L. A., and G. V. Lauder. 2001. Aquatic prey capture in ray-finned fishes: a century of progress and new directions. *J. Morphol.* 248:99–119.
- Fontana, W., and P. Schuster. 1998. Shaping space: the possible and the attainable in RNA genotype-phenotype mapping. *J. Theor. Biol.* 194:491–515.
- Foote, M. 1998. Contingency and convergence. *Science* 280: 2068–2069.
- Garland, T. J. 2003. Selection experiments: an under-utilized tool in biomechanics and organismal biology. Pp. 23–56 in V. L. Bels, J.-P. Gasc, and A. Casinos, eds. *Vertebrate biomechanics and evolution*. The Cromwell Press, Trowbridge, U.K.
- Gatz, A. J., Jr. 1979. Community organization in fishes as indicated by morphological features. *Ecology* 60:711–718.
- Gould, S. J. 1985. The paradox of the first tier: an agenda for paleobiology. *Paleobiology* 11:2–12.
- . 1989. *Wonderful life: the Burgess Shale and the nature of history*. Norton, New York.
- Grubich, J. R. 2000. Crushing motor patterns in drum (Teleostei: Sciaenidae): functional novelties associated with molluscivory. *J. Exp. Biol.* 203:3161–3176.
- Hansen, T. F., and E. P. Martins. 1996. Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution* 50: 1401–1417.
- Hernandez, L. P., and P. J. Motta. 1997. Trophic consequences of differential performance: ontogeny of oral jaw-crushing performance in the sheepshead, *Archosargus probatocephalus* (Teleostei, Sparidae). *J. Zool. (Lond)* 343:737–756.
- Hoffman, A. A., and F. M. Cohan. 1987. Genetic divergence under uniform selection. III. Selection for knockdown resistance to ethanol in *Drosophila pseudoobscura* populations and their replicate lines. *Heredity* 58:425–433.
- Housworth, E. A., and E. P. Martins. 2001. Random sampling of constrained phylogenies: conducting phylogenetic analyses when the phylogeny is partially known. *Syst. Biol.* 50:628–639.
- Hulsey, C. D., and P. C. Wainwright. 2002. Projecting mechanics into morphospace: disparity in the feeding system of labrid fishes. *Proc. R. Soc. Lond. B Biol. Sci.* 269:317–326.
- Huskey, S. H., and R. G. Turingan. 2001. Variation in prey-resource utilization and oral jaw gape between two populations of large-mouth bass, *Micropterus salmoides*. *Environ. Biol. Fishes* 61: 185–194.
- Koehl, M. A. R. 1996. When does morphology matter? *Annu. Rev. Ecol. Syst.* 27:501–542.
- Korona, R. 1996. Genetic divergence and fitness convergence under uniform selection in experimental populations of bacteria. *Genetics* 143:637–644.
- Larson, A., and J. B. Losos. 1996. Phylogenetic systematics of adaptation. Pp. 187–220 in M. R. Rose and G. V. Lauder, eds. *Adaptation*. Academic Press, San Diego, CA.
- Lauder, G. V. 1983. Functional and morphological bases of trophic specialization in sunfishes. *J. Morphol.* 178:1–21.
- Lenski, R. E., and M. Travisano. 1994. Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. *Proc. Natl. Acad. Sci. USA* 91:6808–6814.
- Liem, K. F., and L. S. Kaufman. 1984. Intraspecific macroevolution: functional biology of the polymorphic cichlid species *Cichlosoma minkleyi*. Pp. 203–215 in A. A. Echelle and I. Kornfield, eds. *Evolution of fish species flocks*. Univ. of Maine at Orono Press, Orono, ME.
- Losos, J. B. 1990. Ecomorphology, performance capability, and scaling of West Indian anolis lizards: an evolutionary analysis. *Ecol. Monogr.* 60:369–388.
- Losos, J. B., T. R. Jackman, A. Larson, K. de Queiroz, and L. Rodriguez-Schettino. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. *Science* 279: 2115–2118.
- Lovette, I. J., E. Bermingham, and R. E. Ricklefs. 2002. Clade-specific morphological diversification and adaptive radiation in Hawaiian songbirds. *Proc. R. Soc. Lond. B Biol. Sci.* 269:37–42.
- Martins, E. P. 1996. Conducting phylogenetic comparative studies when the phylogeny is not known. *Evolution* 50:12–22.
- Muller, M. 1987. Optimization principles applied to the mechanism of neurocranial levation and mouth bottom depression in bony fishes (Halecostomoi). *J. Theor. Biol.* 126:343–368.
- . 1996. A novel classification of planar four-bar linkages and its application to the mechanical analysis of animal systems. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351:689–720.
- Norton, S. F. 1995. A functional approach to ecomorphological patterns of feeding in cottid fishes. *Environ. Biol. Fishes* 44: 61–78.
- Pagel, M. 1994. The adaptationist wager. Pp. 29–52 in P. Eggleton and R. Vane-Wright, eds. *Phylogenetics and ecology*. Academic Press, London.
- Ralston, K. R., and P. C. Wainwright. 1997. Functional consequences of trophic specializations in pufferfishes. *Funct. Ecol.* 11:43–52.
- Ricklefs, R. E., and D. B. Miles. 1994. Ecological and evolutionary inferences from morphology: an ecological perspective. Pp. 13–41 in P. C. Wainwright and S. M. Reilly, eds. *Ecological morphology*. Univ. of Chicago Press, Chicago.
- Ross, S. M. 1983. *Stochastic processes*. John Wiley, New York.
- Schuster, P., and W. Fontana. 1999. Chance and necessity in evolution: lessons from RNA. *Physica D* 133:427–452.
- Schuster, P., W. Fontana, P. F. Stadler, and I. Hofacker. 1994. From sequences to shapes and back: a case study in RNA secondary structures. *Proc. R. Soc. Lond. B Biol. Sci.* 255:279–284.
- Stadler, B. M. R., P. F. Stadler, G. P. Wagner, and W. Fontana. 2001. The topology of the possible: formal spaces underlying patterns of evolutionary change. *J. Theor. Biol.* 213:241–274.
- Travisano, M., and R. E. Lenski. 1996. Long-term experimental evolution in *Escherichia coli*. IV. Targets of selection and the specificity of adaptation. *Genetics* 143:15–26.
- Travisano, M., A. J. Mongold, A. F. Bennet, and R. E. Lenski. 1995. Experimental tests of the role of adaptation, chance, and history in evolution. *Science* 267:87–90.
- Van Leeuwen, J. L., and M. Muller. 1984. Optimum sucking techniques for predatory fish. *Trans. Zool. Soc. Lond.* 37:137–169.
- Wainwright, P. C. 1987. Biomechanical limits to ecological performance mollusc-crushing by the Caribbean hogfish *Lachnolaimus maximus* (Labridae). *J. Zool. (Lond)* 213:283–298.
- Wainwright, P. C., and D. R. Bellwood. 2002. Ecomorphology of feeding in coral reef fishes. Pp. 33–55 in P. F. Sale, ed. *Coral reef fishes: dynamics and diversity in a complex ecosystem*. Academic Press, Orlando, FL.
- Wainwright, P. C., D. R. Bellwood, M. W. Westneat, J. R. Grubich, and A. S. Hoey. 2004. A functional morphospace for the skull of labrid fishes: patterns of diversity in a complex biomechanical system. *Biol. J. Linn. Soc. In press*.
- Warheit, K. I., J. D. Forman, J. B. Losos, and D. B. Miles. 1999. Morphological diversification and adaptive radiation: a comparison of two diverse lizard clades. *Evolution* 53:1226–1234.

- Werner, E. E. 1974. The fish size, prey size, handling time relation and some implications. *J. Fish. Res. Board Can.* 31:1531–1536.
- Westneat, M. W. 1990. Feeding mechanics of teleost fishes (Labridae): a test of four-bar linkage models. *J. Morphol.* 205: 269–295.
- . 1991. Linkage mechanics and evolution of the unique feeding mechanism of *Epibulus insidiator* (Labridae, Teleostei). *J. Exp. Biol.* 159:165–184.
- . 1994. Transmission of force and velocity in the feeding mechanisms of labrid fishes. *Zoomorphology (Berl)* 114: 103–118.
- . 1995. Feeding, function, and phylogeny: analysis of historical biomechanics in labrid fishes using comparative methods. *Syst. Biol.* 44:361–383.
- . 1999. The living marine resources of the western central Pacific: FAO species identification sheets for fishery purposes. Family Labridae. Pp. 3381–3467. Food and Agriculture Organization of the United Nations, Rome.
- Westneat, M. W., and P. C. Wainwright. 1989. The feeding mechanism of the sling-jaw wrasse, *Epibulus insidiator* (Labridae; Teleostei): evolution of a novel functional system. *J. Morphol.* 202:129–150.
- Wiens, J. A., and J. T. Rotenberry. 1981. Habitat associations and community structure of birds in shrubsteppe environments. *Ecol. Monogr.* 51:21–42.
- Winemiller, K. O. 1991. Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions. *Ecol. Monogr.* 61:343–365.

Corresponding Editor: J. Wiens