

TESTING THE RELATIONSHIP BETWEEN MORPHOLOGICAL AND MOLECULAR RATES OF CHANGE ALONG PHYLOGENIES

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Abstract.—Molecular evolution has been considered to be essentially a stochastic process, little influenced by the pace of phenotypic change. This assumption was challenged by a study that demonstrated an association between rates of morphological and molecular change estimated for “total-evidence” phylogenies, a finding that led some researchers to challenge molecular date estimates of major evolutionary radiations. Here we show that Omland’s (1997) result is probably due to methodological bias, particularly phylogenetic nonindependence, rather than being indicative of an underlying evolutionary phenomenon. We apply three new methods specifically designed to overcome phylogenetic bias to 13 published phylogenetic datasets for vertebrate taxa, each of which includes both morphological characters and DNA sequence data. We find no evidence of an association between rates of molecular and morphological rates of change.

Key words.—Maximum likelihood, molecular clock, node density effect, phylogenetic independence, relative rates, substitution rate.

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The relationship between rates of phenotypic evolution and genetic change has been a matter of debate for many decades, but in practice the rate of molecular evolution is considered to be effectively disassociated from rate of morphological change. This assumption was challenged by a study of phylogenies for which both morphological characters and molecular data were available (Omland 1997). A comparison of morphological and molecular branch lengths for eight phylogenies, covering a broad range of taxa from ducks to dandelions, revealed a significant association between rates of molecular and morphological change, both for the tips of the phylogenies (branches leading to terminal taxa) and for root-to-tip pathways for each species. This association has been interpreted as indicating a link between the rate of morphological evolution and rate of molecular evolution (e.g., Omland 1997; Conway Morris 1998; Lee 1999). This is a surprising claim, because neither molecular evolutionary theory, experimental studies, nor observation from molecular phylogenies support a close association between morphological and molecular rates of change (see Bromham and Hendy 2000).

Selection for a particular adaptation can prompt localized increases in substitution rates in genes associated with that trait (Gillespie 1991). But any given morphological change is likely to affect only a handful of nucleotide sites in one or a few genes, representing an almost insignificant proportion of the genome. Furthermore, much of the observed molecular change is unlinked to adaptive evolution, occurring at nucleotide sites that do not affect protein or RNA products or causing molecular changes that have no appreciable affect on fitness (Kimura 1983). So, if there is a link between morphological and molecular rates of change, it must be due not to a direct link between adaptation and genetic change, but

to a more general, genomewide process, either directly (e.g., higher mutation rate producing more novel advantageous alleles) or indirectly (e.g., effect of population size on rate of change; Omland 1997). Such a relationship could have serious implications for the way molecular data is used in evolutionary biology. For example, it has been suggested that metazoan lineages underwent an explosive burst in rates of morphological evolution in the early Cambrian and that this drove high molecular rates, making molecular dates of the “Cambrian explosion” divergences between metazoan phyla unreliable (Vermeij 1996; Conway Morris 1998; Knoll and Carroll 1999; Lee 1999; Valentine et al. 1999).

However, Omland’s relationship may be an artifact of the methods used to measure rates of change along phylogenies, rather than reflecting an underlying evolutionary process. There are a number of potential biases in comparing morphological and molecular branch lengths that could cause a spurious association: (1) estimates of amount of morphological and molecular change along any given branch are non-independent because they share the confounding variable of time; (2) both morphological and molecular branch lengths may be subject to similar measurement biases; and (3) phylogenetically independent comparisons are essential to avoid artifactual associations.

Omland (1997) was aware of these problems and attempted to minimize their impact on his analysis. However, we believe that the problem of phylogenetic nonindependence was not removed by his methodology and that it may have resulted in an artifactual association between morphological and molecular rates. Comparison of amount of morphological and molecular change along branches of a phylogeny is problematic because they share the confounding factor of time (Omland 1997). Even if morphological and molecular rates are

unlinked, it is expected that the deeper the divergence, the more substitutions and morphological changes will have accumulated. Because a long branch is likely to have both more morphological changes and more molecular substitutions than a shorter branch, an artifactual association between morphological and molecular change may be caused by their covariation with time. By comparing root-to-tip pathways, summing branch lengths from the base of the tree to the tips, Omland ensured that he compared lineages of the same age. But this approach introduced two other sources of bias: node density effect and phylogenetic nonindependence.

Parsimony infers the minimum number of changes along any given branch. Long unbroken branches will tend to be underestimated by parsimony, because they have more sites that have undergone multiple hits that cannot be directly reconstructed. Adding taxa that break up long branches (increasing the density of nodes) allows more state changes to be inferred, so the amount of change estimated for a lineage is expected to rise with the number of intersecting lineages (Sanderson 1990). If both morphological and molecular branch lengths are estimated by parsimony, then both morphological and molecular rates will be underestimated for long unbroken branches (e.g., species-poor clades), generating an apparent association between molecular and morphological rates. Omland (1997) addressed the problem of node density by using the residuals of a least-squares regression of total path length against node sums (number of nodes separating a terminal taxon from the root); however, this approach is compromised by the use of nonindependent datapoints in the regression. When root-to-tip path lengths are used, the same internal branches contribute to multiple datapoints. This increases the apparent degrees of freedom of the test, artificially inflating the power of the regression. Furthermore, branches lower in the tree will have an undue influence on the inferred relationship.

Comparisons between lineages can only be considered statistically independent if their paths neither meet nor cross on a phylogeny (Harvey and Purvis 1991). Because of the hierarchical nature of phylogenies, most of the root-to-tip pathway of one species will be shared with other species in the phylogeny. Using root-to-tip pathways for all species therefore does not satisfy statistical independence because all but the terminal branches will contribute to more than one datapoint. Similarly, the clade-contrast method (Omland 1997) uses a series of overlapping nested average branch lengths, such that some branches in the phylogeny contribute to more than one datapoint. Using only terminal taxa (tips) in an analysis of rates avoids counting any internodes twice, but is subject to a phylogenetic bias specific to the estimation of rates of morphological change: the underestimation of tip branch lengths. Morphological datasets generally do not include unique (autapomorphic) changes that only occur in a single species, because they are cladistically uninformative (see Bryant 1995; Yeates 1992). Thus, the length of the tips of the phylogeny will be consistently underestimated from morphological data.

We have re-examined the relationship between morphological and molecular rates, using 13 published vertebrate systematic datasets including both DNA sequence and morphological data (Table 1). We apply three new methods aimed

at overcoming phylogenetic bias. Because we find no evidence of a link between morphological and molecular rates for these datasets, we conclude that the association noted by Omland (1997) is likely to be due to phylogenetic bias, rather than being indicative of an underlying association between rates of morphological and molecular evolution.

METHODS

Data

Systematic datasets for vertebrate taxa were selected from the literature that contained sufficient morphological data (more than 30 characters) and DNA sequence data (generally more than 600 nucleotides) for at least seven taxa (Table 1). Phylogenetic overlap was avoided by ensuring no lineages were included in more than one dataset (see legend to Table 1). Sequences were aligned by eye using Se-AL (Rambaut 1996), and any saturated regions that could not be confidently aligned (primarily from 12S rRNA) were excluded from the analysis. For datasets with multiple gene sequences, sequences were concatenated into a single alignment and analyzed together.

In addition to the 13 vertebrate datasets in Table 1, we have reanalyzed three of the phylogenies presented in Omland (1997; Table 2). We were unable to analyze all eight of the datasets in Omland, because our methods are not applicable to restriction data and some of the sequence data was not available on GenBank.

Estimation of Rates

Three new methods were applied to comparing rates of morphological and molecular change along phylogenies. The first step for all three methods was the construction of a phylogeny from DNA sequence data using maximum likelihood. Molecular phylogenies were constructed for each of the 13 datasets listed in Table 1 using PAUP* (ver. 4.0b3a, Swofford 1999) with an HKY + Γ model of nucleotide substitution (Hasegawa et al. 1985; Yang 1994) with transition:transversion ratio (ti:tv) and gamma shape parameter (α) estimated from the data. This model allows for variation in substitution rates across sites, base frequency bias, and transition-transversion bias, yet is both computationally tractable and efficient. Heuristic searches were conducted using the TBR (tree bisection and reconnection) branch-swapping algorithm.

For each of the methods described below, we estimated both morphological and molecular rates on the phylogenetic topology inferred from molecular data. For the first method, phylogenetic analysis of rate estimates (PARE), rates were estimated over the whole phylogeny. For other two methods (average phylogenetic path length estimates [APPLE] and greatest rate across pairwise estimates [GRAPE]) only pairs of sister clades that are consistent with both molecular and morphological phylogenies were used. Molecular rates were estimated using maximum likelihood (HKY + Γ , using the values for α and ti:tv estimated for the whole phylogeny, as described above). Maximum likelihood overcomes a fundamental problem of using parsimony to estimate branch length, which is that parsimony cannot infer more than one state

TABLE 1. Phylogenetic datasets used in this study, with the number of species included in this analysis, DNA sequences used (cytochrome *b* [cytb], cytochrome oxidase subunits I and II [COI, COII], NADH subunits 1, 2, and 4 [ND1, ND2, ND4], 12S and 16S ribosomal RNA [12S, 16S] and transthyretin intron 1 [transthyretin]), the length of the concatenated alignment, number of morphological characters (morph), and source of the data. *Abronia*, *Sceloporus*, *Meroles*, and lacertids are all groups of lizards. To avoid phylogenetic overlap between datasets, the following taxa were deleted: *Canis lupus* and *Vulpes vulpes* from carnivores; *Bos taurus*, *Camelus dromedarius*, *Tragulus napu*, *Tayassu tajacu* from cetaceans; *Sceloporus graciosus*, *Oplurus cuvieri*, *Sauromalus obesus*, *Dipsosaurus dorsalis*, *Sator angustus* from Iguania; *Sceloporus grammicus* and *Phrynosoma texanum* from iguanines; *Lacerta lepida*, *Meroles knoxii*, *Meroles ctenodactylus*, *Pedioplanis burchelli*, *Pedioplanis undata*, *Heliobolus spekkii* from lacertids; *Petrosaurus thalassinus*, *Petrosaurus mearnsi*, *Uta palmeri*, *Uta stansburiana*, and all *Urosaurus* species from *Sceloporus*.

Phylogeny	No. of species	Sequence	Length	Morph	Source
<i>Abronia</i>	15	cytb, 12S	720	30	Chippindale et al. (1998)
Artiodactyla	11	cytb, 12S	2000	40	Montgelard et al. (1998)
Canids	13	cytb, COI, COII	2000	57	Wayne et al. (1997)
Carnivora	15	transthyretin, cytb, 12S	2475	64	Flynn and Nedbal (1998)
Cetacea	22	12S, 16S, cytb	1310	207	Messenger and McGuire (1998)
Crocodylians	10	12S	240	164	Brochu (1997)
<i>Iguania</i>	17	ND1, ND2, COI	900	67	Schulte et al. (1998)
Iguanines	15	ND4	740	90	Sites et al. (1996)
Lacertids	46	12S, cytb, 16S	895	98	Harris et al. (1998b)
<i>Meroles</i>	10	12S, 16S	620	110	Harris et al. (1998a)
Primates	14	cytb	1000	125	Yoder et al. (1996)
<i>Sceloporus</i>	64	12S, 16S	790	196	Wiens and Reeder (1997)
Turtles	23	12S, cytb	1190	115	Shaffer et al. (1997)

change per character per branch. The longer the branch, the more likely that any given character will have changed more than once. Because parsimony cannot infer multiple hits, it will increasingly underestimate the amount of change on longer branches. This is referred to as node density effect, because paths in the phylogeny that have fewer internal nodes (branch points) will be more poorly estimated, whereas increasing the number of nodes (intersecting branches) allows parsimony to infer more changes per path. Because maximum likelihood can reconstruct multiple state changes per character per branch, it is more robust to node density effect than parsimony. There is currently no tractable method for estimating morphological rates using maximum likelihood, so morphological rates were estimated using parsimony: Minimum number of morphological changes per edge were inferred, given a topology estimated from molecular data, using the parsimony criterion with accelerated transformation as implemented in PAUP* (Swofford 1999).

If both morphological and molecular rates are estimated using parsimony, both will tend to be underestimated on long branches due to node density effect, which may generate a spurious association between morphological and molecular rates. Because maximum likelihood is robust to node density, long branches will not have artificially lower molecular rates. So, although morphological rates are estimated using parsimony, the use of maximum likelihood to estimate molecular rates should overcome the problem of shared measurement

bias due to node density effect (i.e., longer branches may have artificially low morphological rates, but they should not have artificially low molecular rates).

Phylogenetic Analysis of Rate Estimates

We used Sanderson's (1997) rate-smoothing method to produce a molecular tree for which node heights correspond to a relative timescale. Nonparametric rate smoothing finds the minimum amount of heritable rate variation required to convert the observed tree with branch lengths in units of substitutions into an ultrametric tree with branch lengths given as relative time. By dividing the observed branch lengths by these times, an estimate of relative rate can be obtained for each branch. These times are also used to estimate relative rates for each morphological branch length. This method allowed us to directly compare rates of molecular and morphological change along each branch of the phylogeny, removing the confounding factor of time. Each branch contributed one datapoint to a correlation analysis between morphological and molecular rates (Fig. 1). Because we expect the rate of morphological evolution to be poorly estimated for tips of the phylogeny, we performed this analysis both with tips included and tips excluded.

Average Phylogenetic Path Length Estimates

We compared average morphological and molecular path lengths along phylogenetically independent sister groups for

TABLE 2. Phylogenetic datasets analyzed by Omland (1997) that have been reanalyzed using three new phylogenetic methods. Because these methods apply only to sequence data, we are unable to include all of the eight datasets in Omland (1997), either because they used restriction site data or because the sequence data was not available on GenBank.

Phylogeny	No. of species	Sequence	Length	Morph	Source
Beetles	11	COI, 16S	865	88	Funk et al. (1995)
Birches	7	<i>rbcL</i>	1523	35	Bousquet et al. (1992)
Echinoids	10	28S	375	81	Smith et al. (1992)

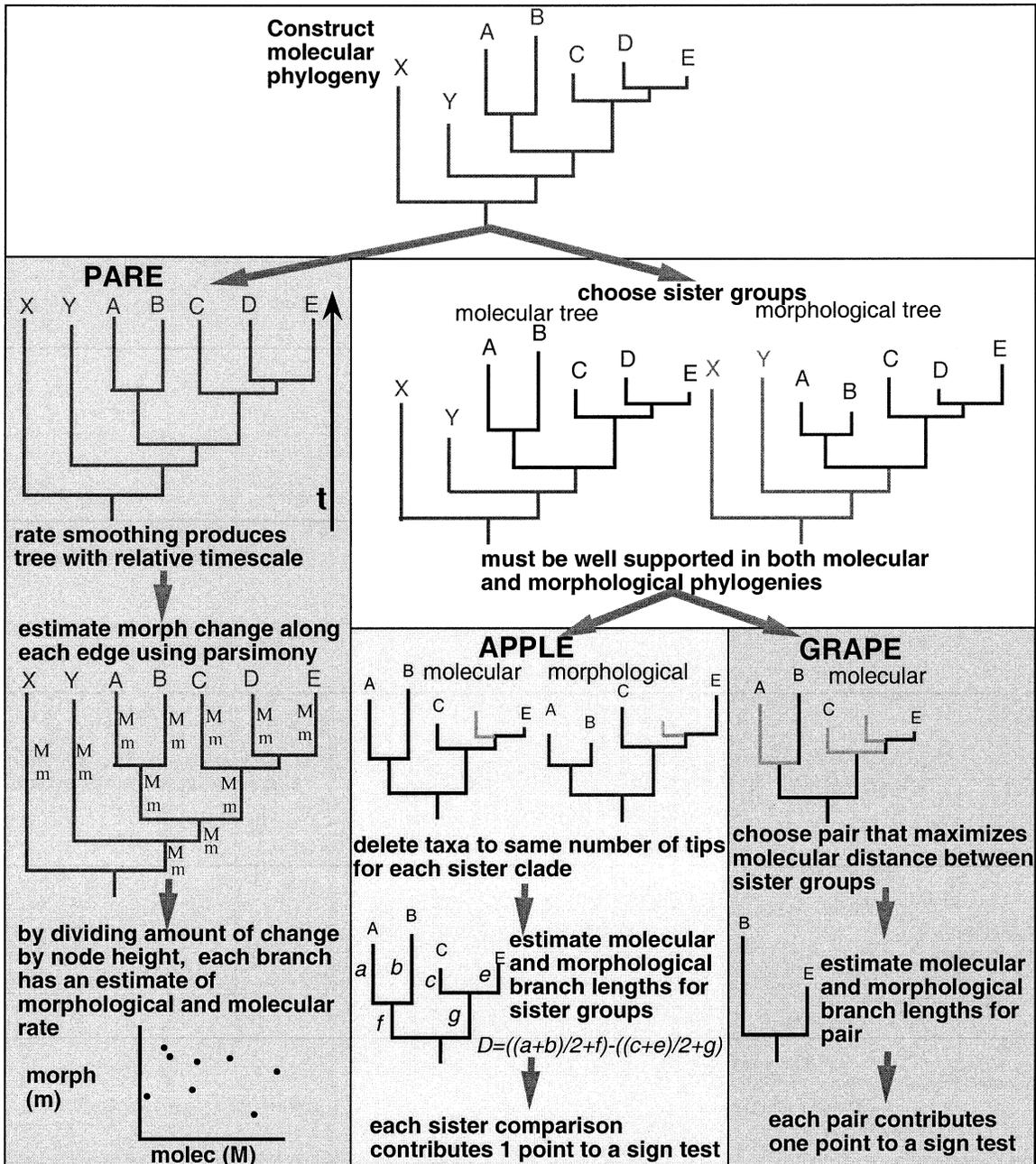


FIG. 1. Methods used in this study. For each of the phylogenetic datasets listed in Table 1, a molecular phylogeny was inferred using maximum likelihood. For PARE, the topology was used to infer a rate-smoothed tree for which node height represents a relative timescale. Rates of morphological and molecular change can then be compared for each branch of the phylogeny. For both APPLE and GRAPE, a pair of sister clades was chosen, which share a common basal node and are therefore the same age. For APPLE, taxa were pruned to give the same number of taxa either side of the basal node. Morphological and molecular methods were estimated on this pruned phylogeny, and average path lengths were calculated for each sister clade. For GRAPE, only one species from each sister clade was selected to maximize the difference in molecular branch length between the two clades; see Methods for details.

each phylogeny. Sister clades share a common basal divergence and are therefore the same age, allowing direct comparison of amount of morphological and molecular change on each sister clade. For each phylogeny, an appropriate pair of sister clades was selected to satisfy four criteria: (1) the node that defined the comparison was well supported: we accepted only those sister groups that were compatible with both the original published molecular and morphological

phylogenies, and our reconstructed maximum-likelihood phylogeny; (2) the node defining the sister groups was as deep as possible in the tree (to minimize the contribution of tips to the path length); (3) the sister groups chosen contained the maximum number of lineages on either side of the sister node; and (4) all sister groups were chosen to be phylogenetically independent. Sister groups chosen are listed in Table 3. Note that because sister groups must be compatible with

TABLE 3. Average phylogenetic path length estimates (APPLE) for molecular data (estimated by maximum likelihood) and morphological data (estimated by parsimony) for each of the two sister groups chosen from the studies listed in Table 1 (with three phylogenetically independent sister groups chosen from the turtle phylogeny). Sign test (8/15, $P = 0.5$) indicates no association between morphological and molecular rates.

Sister groups		Molec1	Molec2	Morph1	Morph2	
<i>Abronia</i>	(<i>Abronia vasconcelosii</i> , <i>A. campbelli</i>), (<i>A. lythrochila</i> , <i>A. fimbriata</i>)	0.0040	0.0155	1.0	5.0	+
Artiodactyla	(<i>Tragulus napu</i> , <i>Cervus nippon</i>), (<i>Camelus bactrianus</i> , <i>Lama guanicoe</i>)	0.0715	0.0271	4.0	0.0	+
Canids	(<i>Canis lupus</i> , <i>Cuon alpinus</i> , <i>Lycaon pictus</i>), (<i>Pseudalopex sechurae</i> , <i>P. culpaeus</i> , <i>Speothos venaticus</i>)	0.0738	0.0123	5.0	1.0	+
Carnivora	(<i>Ursus arctos</i> , <i>Callorhinus ursinus</i> , <i>Zalophus californianus</i> , <i>Erignathus barbatus</i>), (<i>Procyon lotor</i> , <i>Potos flavus</i> , <i>Mustela frenata</i> , <i>Lontra longicaudis</i>)	0.0360	0.0358	4.0	1.3	+
Cetacea	(<i>Ziphius cavirostris</i> , <i>Mesoplodon europaeus</i> , <i>M. peruvianus</i>), (<i>Delphinus delphis</i> , <i>Lissodelphis peronii</i> , <i>Phocoena phocoena</i>)	0.0333	0.0624	2.4	10.0	+
Crocodylians	(<i>Caiman latirostris</i> , <i>Paleosuchus trigonatus</i>), (<i>Alligator sinensis</i> , <i>A. mississippiensis</i>)	0.0792	0.0498	3.0	15.5	–
<i>Iguania</i>	(<i>Chamaeleo fischeri</i> , <i>Leiolepis belliana</i>), (<i>Crotaphytus collaris</i> , <i>Phrynosoma douglassi</i>)	0.4943	0.3542	5.5	10.0	–
Iguanines	(<i>Ctenosaura hemilopha</i> , <i>C. similis</i> , <i>Amblyrhynchus cristatus</i> , <i>Conolophus subcristatus</i>), (<i>Iguana iguana</i> , <i>I. delicatissima</i> , <i>Sauromalus obesus</i> , <i>S. varius</i>)	0.0831	0.0527	11.3	0.8	+
Lacertids	(<i>Lacerta oxycephala</i> , <i>L. chlorogaster</i> , <i>L. horvathi</i> , <i>L. bonnali</i> , <i>L. princeps</i> , <i>L. danfordi</i> , <i>Algyroides marchi</i> , <i>Podarcis muralis</i> , <i>P. taurica</i> , <i>Takydromus septentrionalis</i>), (<i>Acanthodactylus gongrorhynchatus</i> , <i>A. cantoris</i> , <i>A. schmidtii</i> , <i>Poromera fordii</i> , <i>Mesalina adramitana</i> , <i>M. guttulata</i> , <i>Ichnotropis squamulosa</i> , <i>Tropidosaura gularis</i> , <i>Adolfus africanus</i> , <i>A. jacksoni</i>)	0.0751	0.1138	1.4	4.3	+
<i>Meroles</i>	(<i>Meroles suborbitalis</i> , <i>M. anchietae</i>), (<i>Pedioplanis burchelli</i> , <i>P. undata</i>)	0.0338	0.0390	23.0	12.0	–
Primates	(<i>Eulemur fulvus collaris</i> , <i>E. f. rufus</i> , <i>Hapalemur griseus</i>), (<i>Cheirogaleus major</i> , <i>Mirza coquereli</i> , <i>Microcebus murinus</i>)	0.0799	0.1457	7.5	3.8	–
<i>Sceloporus</i>	(<i>Sceloporus formosus formosus</i> , <i>S. taeniocnemis</i> , <i>S. chrysostictus</i>), (<i>S. hunsakeri</i> , <i>S. orcutti</i> , <i>S. licki</i>)	0.0126	0.0061	23.0	27.3	–
Turtles 1	(<i>Geochelone pardalis</i> , <i>Chinemys reevesii</i> , <i>Heosemys spinosa</i>), (<i>Clemmys marmorata</i> , <i>Graptemys pseudogeographica</i> , <i>Trachemys scripta</i>)	0.0810	0.0560	0.3	0.5	–
Turtles 2	(<i>Staurotypus triporcatus</i> , <i>Kinosternon odoratus</i> , <i>Dermatemys mawii</i>), (<i>Carettochelys insculpta</i> , <i>Lissemys punctata</i> , <i>Apalone spinifera</i>)	0.1244	0.1994	2.8	1.0	–
Turtles 3	(<i>Chelodina longicollis</i> , <i>Phrynops gibbus</i> , <i>Chelus fimbriata</i>), (<i>Podocnemis expansa</i> , <i>Pelomedusa subrufa</i> , <i>Pelusios williamsi</i>)	0.1905	0.1556	1.8	1.0	+

both the morphological and molecular trees, this analysis does not rely on the accuracy of the molecular phylogeny alone.

After selecting phylogenetically independent sister groups (typically one pair of sister clades from each phylogeny), an equal number of lineages was selected at random from each clade (maximizing number of species sampled) to limit node density bias on estimation of branch lengths (Fig. 1). The amount of molecular and morphological change along the sister clades was estimated using maximum likelihood for the molecular data and parsimony for the morphological data. Average path lengths were calculated for both morphological and molecular data for each of the sister clades, by summing average path lengths meeting each node traversing down the tree (see Fig. 1; working average path lengths down the clade avoids counting any branch twice, which is critical for producing statistically independent datapoints). If the sister clade with the greater morphological path length also had the longer molecular path length it was scored as positive comparison. Each sister comparison contributed one datapoint to a sign test. Statistical tests that account for magnitude of differences (Spearman rank correlation and Wilcoxon signed-ranks test) were also performed.

Greatest Rate across Pairwise Estimates

This is a simple relative-rates method, which used only one taxon from each of the sister groups selected for the

APPLE analysis. If there is an association between morphological and molecular rates, then we would be most likely to detect it for the pair of taxa that differ the most in molecular branch length. Therefore, to maximize the chance of detecting an association between morphological and molecular rates, one species from each sister group was chosen such that the difference in molecular branch lengths between the two clades was maximized. All nontarget taxa were pruned from the sister groups (as described above), then the molecular branch lengths for these two lineages were estimated by maximum likelihood and the morphological branch lengths estimated by parsimony. If the member of the pair with the longer molecular branch also had the longer morphological branch, it was scored as a positive comparison in a sign test across all phylogenies.

RESULTS

Phylogenetic Analysis of Rate Estimates

Each phylogeny produced a set of paired observations for molecular and morphological rates, one for each branch (for the with-tips analysis) or for each branch excluding those leading to extant species (no tips, Table 4). To determine whether a parametric statistical test was appropriate, we tested the distribution of the log-transformed data against a normal distribution (using the Shapiro-Wilks test statistic; Sokal and Rohlf 1995). For most of the phylogenies, the estimates

TABLE 4. Results from the phylogenetic analysis of rate estimates (PARE). For each phylogeny, the analysis was conducted on all branches in the phylogeny (with tips) or only on internodes, excluding terminal branches (no tips). Variables recorded are the number of branches included in each dataset (N), and correlation coefficient (r) and P -values (P) for a Spearman rank correlation. Fisher's combined test of P -values is nonsignificant for both with tips ($P = 0.96$) and no tips ($P = 0.88$).

Taxon	With tips			No tips		
	N	r	P	N	r	P
<i>Abronia</i>	25	0.001	1.00	11	-0.050	0.88
Artiodactyla	18	0.112	0.66	8	-0.071	0.87
Canids	16	0.294	0.27	7	0.357	0.43
Carnivora	22	0.020	0.93	10	-0.006	0.99
Cetacea	40	0.283	0.08	19	0.090	0.71
Crocodylians	12	0.210	0.51	5	-1.000	0.00
<i>Iguania</i>	29	0.269	0.16	13	0.160	0.60
Iguanines	24	-0.007	0.97	11	-0.664	0.03
Lacertids	83	0.112	0.31	40	0.054	0.74
<i>Meroles</i>	16	0.368	0.16	7	0.786	0.04
Primates	14	0.464	0.09	6	0.600	0.21
<i>Sceloporus</i>	103	0.201	0.04	46	0.001	0.99
Turtles	44	0.164	0.29	21	0.153	0.51

of morphological rates did not conform to a normal distribution, so we elected to use a nonparametric Spearman rank correlation. Although there were four significant correlations, an inspection of the data shows little indication of a relationship between morphological and molecular rates (Fig. 2). First, the significant correlations were evenly distributed between positive and negative correlations (Table 4): Only positive correlations support Omland's result. Second, all four significant correlations were due to one or few positive outliers in the morphological data (Fig. 2). Third, a meta-analysis across all phylogenies did not indicate any overall trend in the data: Fisher's combined test of P -values (Sokal and Rohlf 1995) was nonsignificant (with tips, $P = 0.96$; no tips, $P = 0.88$). Given these three observations, the results of the PARE analysis do not provide evidence of an association between morphological and molecular rates for these data. The PARE analysis may be influenced by nonindependence due to heritability of rates or by the reliance on inferring molecular branch lengths using rate smoothing (see Discussion). However, the APPLE and GRAPE methods are not subject to these problems, and yet their results are consistent with PARE.

Average Phylogenetic Path Length Estimates and Greatest Rate across Pairwise Estimates

There was no evidence of an association between morphological and molecular rates either for the average phylogenetic path length estimates (APPLE: Table 3) or for the greatest rate across pairwise estimates (GRAPE: Table 5). Each of these analyses used 15 comparisons (one from each phylogeny, with three from the turtle phylogeny). For APPLE, there was an almost even number of positive and negative comparisons (sign test: 8/15, $P = 0.5$). Spearman rank correlations (additive contrasts: $r_s = 0.07$, $P = 0.81$; proportional contrasts: $r_s = 0.25$, $P = 0.38$) and Wilcoxon signed-ranks test ($Z = -1.04$, $P = 0.15$) did not reveal significant associations between morphological and molecular

rates. The sign test for GRAPE was also nonsignificant (10/15, $P = 0.15$).

Data from Omland (1997)

Reanalysis of three datasets from Omland's (1997) study using the three new methods described in this paper revealed no consistent relationship between morphological and molecular rates (Table 6). Of the PARE analyses, only beetles with tips had a significant r -value, and this was due to a large positive outlier in the morphological data. Scatterplots give no indication of a positive trend in any of the six datasets (with tips and no tips for three phylogenies). There are not enough comparisons to perform statistical analyses for the APPLE and PARE results, but there does not appear to be a consistent trend in the data (Table 6).

DISCUSSION

Our analysis of 13 systematic datasets revealed no significant association between rates of morphological and molecular change for these phylogenies. We suggest that the association between morphological and molecular branch lengths noted by Omland (1997) may have been due to phylogenetic bias and therefore does not imply an underlying association between rates of morphological and molecular evolution. Two aspects of Omland's analysis may have led to an association between estimates of molecular and morphological change along phylogenies. First, both the root-to-tip pathways and the clade-averaging approach included non-independent datapoints, which may have artificially inflated the degree of association between morphological and molecular rates. The analysis using only tips limited the use of nonindependent datapoints, but was prone to error in estimation of morphological rates due to the undersampling of autapomorphies in systematic datasets. Second, shared measurement bias arising from the use of parsimony to estimate branch lengths may have led to an artifactual association, because clades with more lineages will tend to have higher estimates of both molecular and morphological branch lengths. Parsimony is primarily a means of inferring phylogenetic topology. Because it does not allow for multiple hits along branches of a phylogeny, parsimony is an inappropriate way of estimating branch length, particularly for molecular data, for which multiple hits will be common.

We have used three new methods aimed at overcoming these phylogenetic biases. Each method has different strengths and weaknesses, but all point to a lack of association between morphological and molecular rates. We have avoided shared measurement bias due to the node density effect by using maximum likelihood to estimate molecular branch lengths—because maximum likelihood is robust to node density effect, this will break the shared measurement bias between morphological and molecular rates and thus should remove this potential source of bias. We further guard against node density effect by comparing sister groups with the same number of lineages in APPLE and GRAPE.

To avoid bias due to including nonindependent datapoints in the analysis, each of our methods ensures that each branch of the phylogeny is included in the analysis only once. Using internodes as independent estimators of the relationship be-

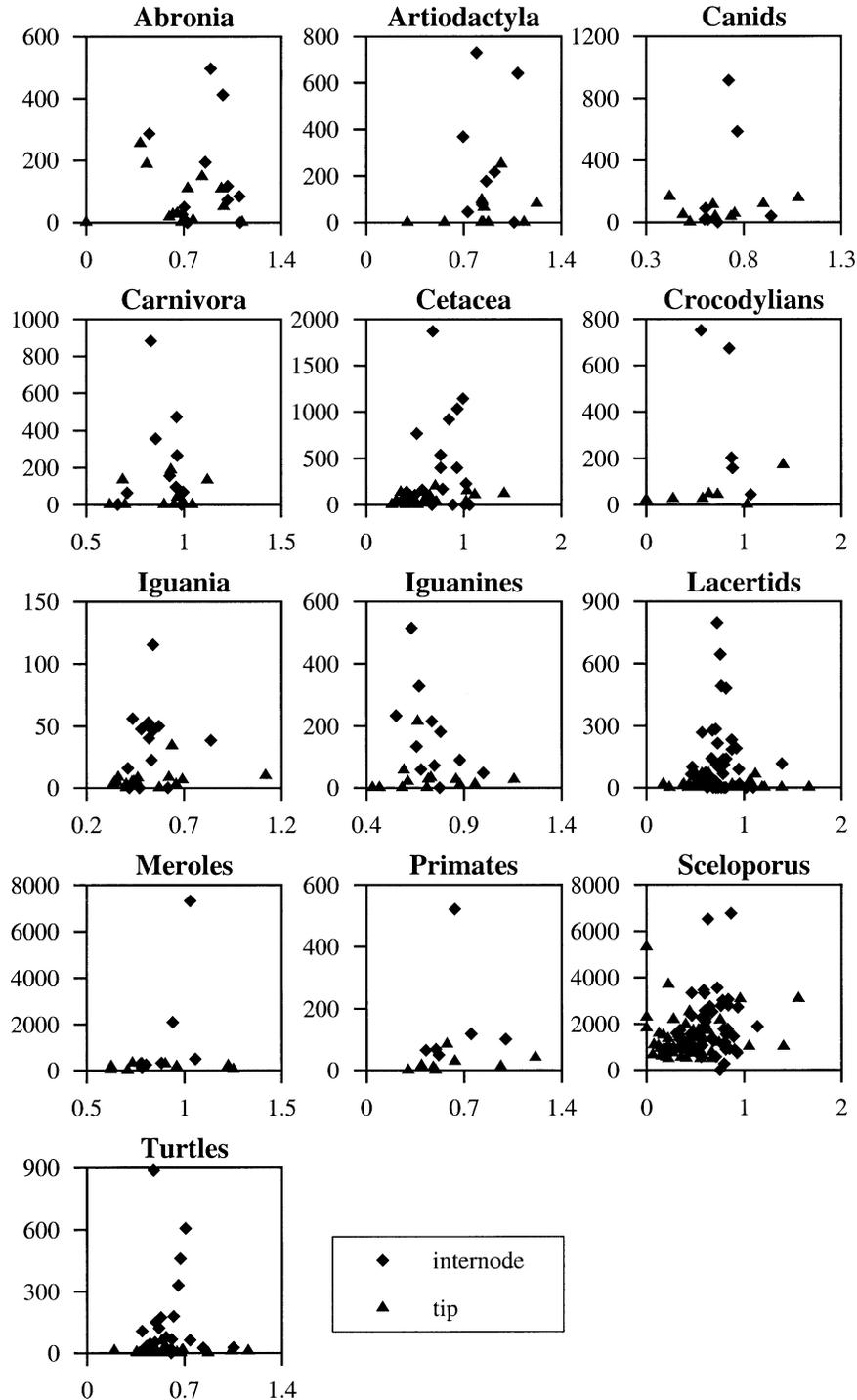


FIG. 2. Morphological rates (y-axis: parsimony steps per unit time) plotted against molecular rates (x-axis: substitutions per site per unit time) estimated using the phylogenetic analysis of rate estimates (PARE) for the datasets listed in Table 1. Each datapoint represents one branch of the phylogeny (diamonds, internodes; triangles, tips). Units of time are relative to node heights on the rate-smoothed phylogeny; see Methods for details.

tween morphological and molecular rates (PARE) may introduce a phylogenetic bias if initial rates for both molecular and morphological change for an internode are inherited from the parent lineage. However, this nonindependence is more likely to promote an association between morphological and molecular rates, yet no association is apparent (Fig. 2). In

any case, APPLE and GRAPE use sister groups chosen conservatively to ensure that none of the comparisons overlap on the phylogeny and therefore are not subject to this phylogenetic bias. Similarly, the use of rate smoothing in PARE might reduce the power to detect a relationship between morphological and molecular rates by accounting for some var-

TABLE 5. Results of greatest rate across pairwise estimates (GRAPE) analysis. One species was chosen from each sister group listed in Table 3 to maximize the difference in molecular branch lengths between the pair. A sign test reveals no significant association between molecular and morphological branch lengths for these pairs (10/15, $P = 0.15$).

Phylogeny	Pair	Molec1	Molec2	Morph1	Morph2		
<i>Abronia</i>	<i>Abronia aurita</i>	<i>Abronia fimbriata</i>	0.0471	0.0291	26	22	+
Artiodactyla	<i>Bos taurus</i>	<i>Lama guanicoe</i>	0.0766	0.0690	18	0	+
Canids	<i>Lycaeon pictus</i>	<i>Cerdocyon thous</i>	0.1346	0.0718	19	5	+
Carnivora	<i>Procyon lotor</i>	<i>Ursus arctos</i>	0.0841	0.0499	4	12	-
Cetacea	<i>Lagenorhynchus albirostris</i>	<i>Ziphius cavirostris</i>	0.1126	0.0893	26	21	+
Crocodylians	<i>Caiman crocodilus</i>	<i>Alligator sinensis</i>	0.1337	0.0449	18	14	+
Iguania	<i>Chamaeleo fischeri</i>	<i>Hoplocercus spinosus</i>	0.9655	0.3645	16	1	+
Iguanines	<i>Ctenosaura palearis</i>	<i>Sauromalus varius</i>	0.1391	0.1160	6	26	-
Lacertids	<i>Acanthodactylus gongrorhynchatus</i>	<i>Podarcis muralis</i>	0.2335	0.0699	32	10	+
<i>Meroles</i>	<i>Meroles reticulatus</i>	<i>Pedioplanis burchelli</i>	0.0926	0.0621	29	19	+
Primates	<i>Microcebus murinus</i>	<i>Lemur catta</i>	0.3354	0.1348	28	20	+
<i>Sceloporus</i>	<i>Sceloporus formosus</i>	<i>Sceloporus hunsakeri</i>	0.0325	0.0103	27	35	-
Turtle 1	<i>Chinemys reevesii</i>	<i>Graptemys pseudogeographica</i>	0.1108	0.0950	2	4	-
Turtles 2	<i>Lissemys punctata</i>	<i>Dermatemys mawii</i>	0.2916	0.1567	16	7	+
Turtles 3	<i>Pelusios williamsi</i>	<i>Phrynops gibbus</i>	0.3629	0.1382	6	14	-

iation in molecular rates as difference in node height (although power may be increased by more efficient use of data because all branches of the phylogeny are included), but this will not be a problem for APPLE or GRAPE, which do not use rate smoothing. Therefore, the failure to find an association between morphological and molecular rates for these phylogenies using any of the three methods suggests there is no general, observable relationship between rates of morphological and molecular change along phylogenies.

Failure to detect a relationship between morphological and molecular rates along these phylogenies does not necessarily imply that molecular evolution is unlinked to rates of morphological evolution. It may simply be that phylogenetic datasets are not the most appropriate way of exploring this relationship. Genes directly associated with measurable phenotypic change will make up a only a tiny proportion of the genome and are unlikely to be included in phylogenetic datasets. The DNA sequences used in phylogenetics, commonly “housekeeping” genes associated with universal processes in metabolism and DNA replication, will only reflect the relationship if rate of morphological evolution is linked to a general, genomewide phenomenon, such as increase in mutation rate to produce more novel alleles, or effect of population size on substitution rate. Similarly, the morphological characters used in phylogenetics are chosen specifically to

delineate taxonomic groups (often discrete novelties) and may not be the most appropriate characteristics for measuring the rate of adaptive phenotypic change. Another way of testing for a link between morphological and molecular rates of change would be comparison of morphological disparity against broad-scale genomic change. There are many ways of measuring morphological disparity, such as the phenetic (principal component analysis of morphological character space) and cladistic analyses (number of character transitions from the basal node of the tree to each terminal taxon) developed by M. Wills and colleagues (Briggs et al. 1992; Wills et al. 1994; Fortey et al. 1996; Wills 1998). This approach could be extended to ecological specialization: for example, Cotgreave and Harvey (1994) used the length of the description of each family in Austin’s *Birds of the World* (1961) as a proxy variable for the amount of variation in appearance and habit in bird families. DNA-DNA hybridization data may prove particularly useful in the study of any association between morphological disparity and rate of molecular evolution because it surveys the entire single-copy nuclear component of the genome. The association between substitution rate and taxonomic diversity—the number of species or higher level taxa per clade—for a DNA sequence for flowering plants (Barraclough et al. 1996; Barraclough and Savolainen

TABLE 6. Results for the three datasets from Omland (1997) analyzed using the three methods described here (Table 2). Phylogenetic analysis of rate estimates (PARE): number of internodes, correlation coefficient, and P -value given for Spearman rank correlation, both including terminal taxa (tips) and excluding terminal taxa (no tips). Average phylogenetic path length estimates (APPLE): average morphological and molecular path lengths for the following sister taxa, with the sign of the contrast. Sister groups used for the APPLE analysis were: beetles (*Ophraella arctica*, *O. artemisiae*, *O. communis*, *O. notulata*), (*O. notata*, *O. cribrata*, *O. conferta*, *O. pilosa*); birches (*Alnus incana*, *Betula papyrifera*), (*Corylus cornuta*, *Ostrya virginiana*); echinoids (*Echinocardium cordatum*, *Spatangus purpureus*, *Brissus unicolor*), (*Psammechinus miliaris*, *Sphaerechinus granularis*, *Lytechinus variegatus*). Greatest rate across pairwise estimates (GRAPE): molecular and morphological branch lengths and the sign of the contrast for the following pairs: beetles (*Ophraella pilosa*, *O. notulata*); birches (*Carpinus caroliniana*, *Betula papyrifera*); echinoids (*Lytechinus variegatus*, *Echinocardium cordatum*).

Phylogeny	PARE (tips)			PARE (no tips)			APPLE				GRAPE					
	N	r	P	N	r	P	Molec1	Molec2	Morph1	Morph2	Molec1	Molec2	Morph1	Morph2		
Beetles	18	0.55	0.02	8	0.62	0.1	0.0397	0.1064	7.38	13.75	+	0.1017	0.0898	20	21	-
Birches	8	0.38	0.35	3	-0.50	0.67	0.0030	0.0040	1.50	5.50	+	0.0137	0.0030	15	6	+
Echinoids	14	-0.24	0.4	5	-0.20	0.75	0.0000	0.0066	4.00	1.75	-	0.0614	0.0113	12	35	-

2001) suggests that the search for “macroevolutionary” correlates of rates of molecular evolution may be profitable.

Although it is commonly taken for granted that molecular evolution proceeds independently of morphological change, it is important that the association between morphological and molecular rates is thoroughly tested. If substitution rates are influenced by the pace of adaptive change, then the results of some molecular clock studies could be misleading. For example, it has been suggested that in “explosive” evolutionary radiations, the molecular clock might run fast due to a greatly accelerated pace of morphological change (Vermeij 1996; Conway Morris 1998; Valentine et al. 1999). Although no mechanism for such a connection is proposed, this hypothesis has been put forward as a possible refutation of the surprisingly old molecular dates for three major adaptive radiations inferred from the fossil record: metazoan phyla in the early Cambrian and mammal and bird families in the early Tertiary. At the broadest taxonomic level, there is undoubtedly an association between adaptation and rates of molecular evolution, for example, rapidly evolving viruses have the greatest rates of DNA sequence evolution. But there is little indication of a more general relationship between morphological and molecular rates: Lineages with high rates of morphological change do not have notably higher rates of molecular change. A close relationship between morphological and molecular rates is unexpected because very little of the genome is directly connected to adaptive change, so most molecular change will be effectively stochastic, hence the expectation of a molecular clock. However, the independence of the molecular clock from adaptive evolution deserves further empirical investigation.

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