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SEQUENCE ALIGNMENT, PARAMETER SENSITIVITY, AND THE PHYLOGENETIC ANALYSIS OF MOLECULAR DATA

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Abstract.—The dependence of the results of molecular phylogenetic sequence analysis (both alignment and cladogram construction) on variation in analytical parameters is examined. Phylogenetic analyses of molecular sequence data are necessarily based on intrinsically unmeasurable parameters such as transition–transversion and alignment gap cost ratios (among others). Procedures for robust and liberal hypothesis choice are proposed using congruence as an optimality criterion. To illustrate and explain this process further, data on arthropod relationships are used. The effects of variation in transversion–transition and gap–change ratio parameters on alignment and phylogeny reconstruction are assessed in light of both taxonomic and character-based congruence measures. [Sequence alignment; sensitivity analysis; arthropods; molecular systematics; phylogeny.]

The phylogenetic analysis of nucleic acid sequences, as with other data, is unavoidably based on explicit and implicit assumptions. At the fore are character transformation models—usually transversion–transition ratios—and the relative cost of alignment-derived sequence gaps. These values are the fulcra of sequence analysis. Simple homogeneous weighting does not avoid the issue of arbitrary, yet crucial, assumptions. Transversion–transition ratios and alignment gap costs are generally not directly measurable. These values are statements of process, and they can only be inferred appropriately from a predetermined phylogenetic pattern. The disturbing circularity of the interaction between the specification of values a priori and their inference a posteriori is a general and central problem in molecular phylogenetic analysis.

One potential solution to the problem of parameter sensitivity has been proposed by Farris (1969; amplified by Carpenter, 1988) through the successive approximations weighting (SAW) procedure. Iteration is used to estimate parameters repeatedly (in this case character weights) by reconstructing phylogeny and using this phylogeny to generate new self-consistent parameter estimates. This process is reprised until stability in inferred weights is achieved. The SAW approach has been extended for character transformation weights by Williams and Fitch (1989). Although an iterative approach is in some sense objective, it will not yield information as to how sensitive the results are to the specific model (set of analysis parameters) the process yields. Iteration is a way to choose some models over others, but it does not tell us how much better these models are. Furthermore, all iterative approaches are to some degree sensitive to the initial conditions (a priori weights) of the analysis.

Even though transversion–transition and gap–change cost ratios are unmeasurable in the absence of a predetermined phylogeny, it is possible to estimate their values through appeal to an external optimality criterion. The most reasonable optimality criterion for phylogenetic analysis must be congruence (whether taxonomic [Nelson, 1979] or character based [Mickevich and Farris, 1981]; but see Miyamoto, 1981, 1985). Without any way of objectively measuring the accuracy of reconstruction, only precision (the agreement among data) can be used to arbitrate among competing hypotheses. This same sort of precision (in the guise of congruence) can be used to assay both the quality and robustness of phylogenetic hypotheses.

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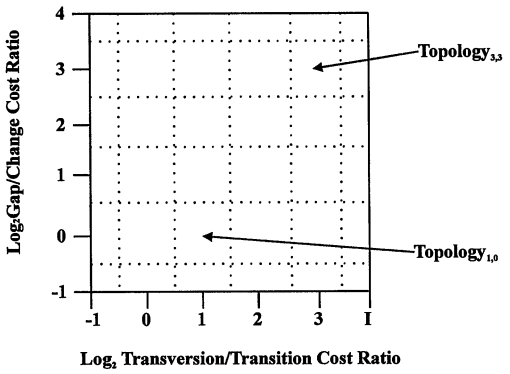

FIGURE 1. The simple two-dimensional analysis space examined here. The axes (gap–change cost ratio and transversion–transition cost ratio) are presented as the logarithm (base 2) of the cost ratios (e.g., 0 = cost ratio of 1). Different locations in the space may yield different phylogenetic results (topologies). I = an infinite transversion–transition cost ratio.

To estimate the sensitivity of an analysis to variation in parameter values, the range of each of the parameters (such as character weight and bias = asymmetry: $i \rightarrow j \neq j \rightarrow i$) must be determined. This range establishes the analysis space of the problem. In this space, all possible combinations of parameter values are present, hence all analytical conclusions are implied. These combinations of values then are sampled and their analytical consequences determined. This process would, in the most general case, involve an n-dimensional hyperspace with each of the n parameters defining an axis bounded by the parameter ranges. In the analyses performed here, the behavior of two variables was investigated: (1) transversion–transition ratio and (2) alignment gap–change cost ratio. These two parameters constitute the axes of a simple analysis space (Fig. 1). The sampling regime would consist of taking parameter pairs (transversion–transition ratio, gap–change cost ratio) from this space, erecting hypotheses of relationship based on these values, and assaying congruence with an external data set.

Even with only the two parameters discussed here, the available analysis universe is infinite. Each of the parameters can, at least numerically, achieve any positive real value. Realistic sampling of such a space would be extremely difficult. Fortunately, constraints on the values of these parameters simplify the situation considerably. The triangle inequality effectively removes most of this space from concern, making credible sampling tractable (although finer grained analysis always will be preferable). The triangle inequality as applied to character evolution constrains character transformation models (Wheeler, 1993). When applied to nucleotide data, three constraints present themselves. First, character transformations must be symmetrical ($i \rightarrow j = j \rightarrow i$, for a bias of zero). Polarities may be specified by outgroups or some other means, but the cost of forward and reverse changes must be identical. Second, the transversion–transition cost ratio must be at a minimum 0.5. Without this limit, transversions could be so cheap as to mediate all change, even in the absence of observed states, which would make the transformation series empirically possible (A $\rightarrow$ C $\rightarrow$ G, when C is unobserved). There is no upper bound for this ratio; transversions may be infinitely more costly than transitions (transitions effectively costing zero). Third, like the transversion–transition ratio, the ratio of the cost of gaps (alignment insertions) must be at least one half the cost of changes (character transformations) and may vary upward without bound (I = infinite, Fig. 1) or else the same unobserved intermediate problem will occur (A $\rightarrow$ gap $\rightarrow$ G, when no gaps are observed in any of the terminals). These constraints limit the analysis space by reducing both the dimensionality (bias is constant at zero) and range of possible values.

Within these theoretical limits, a residuum of possible values exists for the analysis parameters. Here, a plane bounded on two adjacent sides is defined (Fig. 1). Because any and all combinations of parameter values that fall in this plane are possible at least logically, they all (or at least some sample of them) must be examined. To accomplish this sampling, alignment and phylogeny reconstruction must be performed with sufficient combinations of

possible values to represent the behavior of the entire space. This procedure is relatively straightforward (although time consuming). For each point (a combination of transversion–transition and gap–change cost ratios) to be sampled, the sequences are aligned and a phylogeny is reconstructed. Both alignment and phylogeny reconstruction are performed using the same combination of parameter values. At each of these points, some measure of congruence is calculated with respect to some external data set, the variation of which can be used to assay both the most appropriate values for the unmeasurable parameters and the effects of variation in these parameter values on the overall conclusions of the analysis.

If some congruence measure is plotted with respect to the parameter values, a congruence surface is generated. The relief in this surface denotes the areas of relative congruence and incongruence. This surface can be used to estimate the values of the analytical parameters. As with statistical inference, two types of decisions (estimates of parameter values) can be made: best and robust. A best decision is made by choosing the set (or sets) of parameter values at which the optimality criterion is maximized. According to this type of decision, the set of values for the transversion–transition ratio and the gap–change ratio that maximize congruence would be chosen. A robust decision selects a range of parameter values rather than settling on a single set. This range defines a subset of the analysis space in which some statement is supported. For example, an area might be specified in which some group was monophyletic.

Congruence Assay

Most discussions of congruence fall into one of two camps, taxonomic and character. Taxonomic congruence (topology based, usually expressed in terms of cladogram consensus) is concerned with the agreement among the conclusions of phylogenetic analyses (Nelson, 1979; Rohlf, 1982; Bremer, 1990). Shared information may be expressed in terms of the number of commonly supported groups (expressed in terms of resolution of strict consensus; Rohlf, 1982). The comparisons scored here compare the strict consensus of the cladograms generated from the molecular data with that generated from the morphological information. If two analyses each support the same set of groups, they are completely congruent. Here, taxonomic congruence is expressed in terms of the percentage of common groups. Two means of enumerating common groups are employed. In the first, percent shared groups (PSG), only those groups that are demonstrably monophyletic are considered congruent (unresolved and paraphyletic groupings are considered to be incongruent). This definition is derived from the strict consensus procedure. A second and more catholic measure, percent consistent groups (PCG), is also used. The PCG is more closely allied with the combinable component consensus (semistrict) method of Bremer (1990), including unresolved yet noncontradictory groups as congruent. When the PCG values are calculated, unresolved groups contribute 0.5 to the enumerated congruences, whereas monophyletic groups contribute 1. A value of zero implies no shared groups, and a value of 1 denotes complete taxonomic agreement.

Character congruence (Mickevich and Farris, 1981), the second type of congruence, seeks to measure the degree of character conflict among multiple data sets. The statistic of Mickevich and Farris (1981) quantifies the degree of character conflict by measuring the number of extra steps forced upon the individual data sets when they are combined. In this way, the additional conflict created by the combination of the data is assessed separately from that derived from internal character conflict. The value generated is simply the length of the most-parsimonious cladogram(s) derived from the combined data minus the sum of the lengths of the cladograms from the constituent data sets. This number of steps is normalized through division by the length of the combined data cladogram. A value of zero implies complete character congruence, whereas higher val-

TABLE 1. Arthropod and related taxa used in the study by Wheeler et al. (1993).

Mollusca	Cephalopoda	*Loligo pealei*
	Polyplacophora	*Lepidochiton cavernae*
Annelida	Polychaeta	*Glycera* sp.
	Oligochaeta	*Lumbricus terrestris*
	Hirudinea	*Haemopis marmorata*
Onychophora	Peripatoidae	*Peripatus trinitatis*
	Peripatopsidae	*Peripatoides novozealandia*
Chelicerata	Pycnogonida	*Anoplodactylus portus*
	Xiphosura	*Limulus polyphemus*
	Scorpiones	*Centruroides hentzii*
	Uropygi	*Mastogoproctus giganteus*
	Araneae	*Nephila clavipes, Peucetia viridans*
Crustacea	Cirripedia	*Balanus* sp.
	Malacostraca	*Callinectes* sp.
Myriapoda	Chilopoda	*Scutigera coleoptrata*
	Diplopoda	*Spirobolus* sp.
Hexapoda	Zygentoma	*Thermobius* sp.
	Ephemerida	*Heptagenia* sp.
	Odonata	*Libellula pulchella, Dorocordulia lepida*
	Dictyoptera	*Mantis religiosa*
	Auchenorrhyncha	*Tibicen* sp.
	Lepidoptera	*Papilio* sp.
	Diptera	*Drosophila melanogaster*

ues denote increasing degrees of character conflict between the data sets. No topology statement is implied or required. In fact, data sets with zero taxonomic congruence can have 100% character congruence if one data set yields an unresolved bush and the second yields one of its many potential resolutions. The analyses performed here use both approaches to measures of congruence.

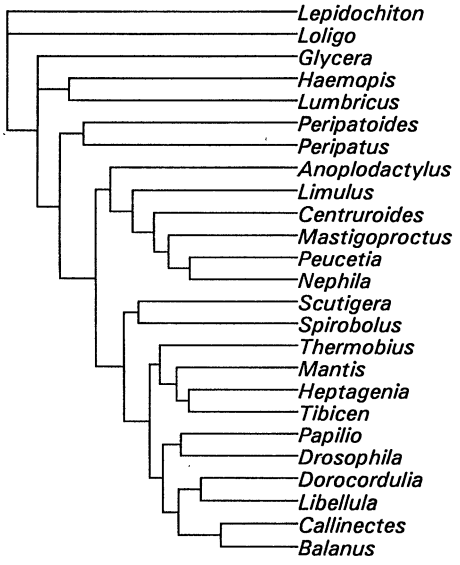

FIGURE 2. Consensus cladogram derived from molecular data of Wheeler et al. (1993). Note the crustacean taxa (*Callinectes* and *Balanus*) within hexapods. The total analysis (including morphological features) placed Crustacea as the sister to Hexapoda + Myriapoda. The cladogram was created with Clados (Nixon, 1992).

METHODS

The central questions in the study of arthropod relationships concern the precise nature of the interrelationships among higher taxa and arthropod monophyly itself (Snodgrass, 1952; Manton, 1964; Weygolt, 1986; Ballard et al., 1992). The data analyzed here (Wheeler et al., 1993) consist of approximately 650 bases of the 18S nuclear ribosomal DNA (18S rDNA) gene, 228 bases of the polyubiquitin locus, and 100 literature-based morphological features.

The molecular data were gathered from each of 25 taxa. The higher level groups (molluscs, roundworms, clitellate roundworms, onychophorans, chelicerates, crustaceans, myriapods, and insects) were each represented by at least two taxa. In each case, the taxa were chosen to be maximally divergent cladistically (Table 1).

The results presented by Wheeler et al. (Fig. 2) rely on a specific model of analysis with the transition–transversion cost ratio

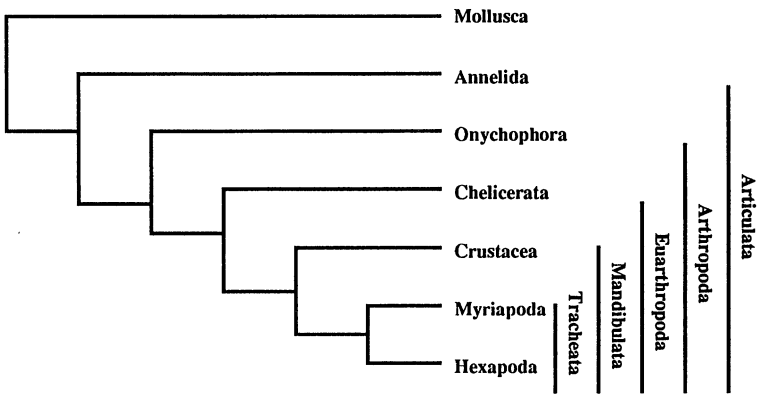

FIGURE 3. Morphology-based cladogram of Wheeler et al. (1993) showing arthropods and related groups (Table 1).

at 1:1 (all base transformations equally costly) and the gap–change cost ratio at 4:1 (although gap information was not included and morphological information was explicitly incorporated). In the present reanalysis, I examined the generality of the results derived from the previous, more limited analysis.

To determine the effects of alignment and character transformation model variation, the complete molecular data were realigned and cladograms reconstructed a total of 36 times. The gap–change ratio varied in a logarithmic fashion, with the cost of gaps from 0.5 to 16 times the cost of a character change ($\log_2$gap/change = −1, 0, 1, 2, 3, 4). For each of these gap–change ratios, six transversion–transition costs were examined. The first five vary as with the gap ratios in a logarithmic manner ($\log_2$transversion/transition = −1, 0, 1, 2, 3), and the sixth model applied zero cost to transitions; hence, the cost ratio was effectively infinite (transversion parsimony). The lower limits on these ratios were established by the strictures of the triangle inequality.

The sequence alignment was performed using the program MALIGN (Wheeler and Gladstein, 1992), which strives to generate alignments that yield parsimonious cladograms, and phylogenetic analysis was performed using Hennig86 (Farris, 1988) and PAUP (Swofford, 1990). In each trial, the identical parameter set was used for both alignment and phylogeny reconstruction. Identical transversion–transition cost ratios were used, and the alignment gap–change cost ratio was converted into a cost factor (character weight) for gaps as character states in the phylogenetic analysis.

RESULTS

For each of the 36 combinations of transversion–transition and gap–change cost ratios, the status of each of the groups (Fig. 3) supported by the morphological matrix was assayed (i.e., whether the group was supported as monophyletic, unresolved, or nonmonophyletic; Table 2). Additionally, the Mickevich and Farris (1981) character incongruence measure was determined, as were the PSG and PCG. These measures were plotted, illustrating their overall agreement (although they differ in several specifics; Fig. 4).

The phylogenetic results were plotted in the analysis space with respect to the congruence measures (Figs. 5, 6), and the surfaces of taxonomic and character congruence were constructed. These surfaces describe the behavior of the analyses with respect to variation in the values of the two parameters examined. The overall congruence attributes for all taxa are examined in this way. Individual higher taxa can be examined to determine in which areas of analysis space they are supported (Fig. 7).

TABLE 2. Sensitivity data for arthropods and related taxonomic groups under various scenarios of analysis (combinations of transversion–transition and gap–change cost ratios).

Trial no.	Tv/Ti[a]	G/C_{max}[b]	Taxonomic groups[c]															PSG[d]	PCG[e]	ME[f]
			Ml	An	Cl	At	On	Eu	Ch	Ec	Ac	Ar	Mn	Cr	Tr	My	Hx			
1	−1	−1	1	0	0	1	1	1	0	0	0	0	0	1	0	1	0	0.40	0.40	1.03
2	−1	0	1	U	1	1	1	1	0	0	0	0	0	1	0	1	0	0.47	0.50	0.47
3	−1	1	1	U	1	1	1	1	0	0	0	0	0	U	0	1	0	0.40	0.50	0.53
4	−1	2	1	U	1	1	1	1	0	0	0	0	0	1	0	1	0	0.47	0.50	0.43
5	−1	3	1	U	1	1	1	1	0	0	0	0	0	1	0	1	0	0.47	0.50	0.32
6	−1	4	1	U	1	1	1	1	0	0	0	0	0	1	0	1	0	0.47	0.50	0.22
7	0	−1	1	1	1	0	1	1	0	0	0	0	0	1	0	1	0	0.47	0.47	1.26
8	0	0	1	U	1	1	1	1	0	0	0	0	0	1	0	1	0	0.47	0.50	0.57
9	0	1	1	U	1	1	1	1	U	U	U	U	U	1	U	1	U	0.47	0.73	0.22
10	0	2	1	U	1	1	1	1	0	0	0	0	0	1	0	1	0	0.47	0.50	0.29
11	0	3	1	U	1	U	1	1	0	0	0	0	0	1	0	1	0	0.40	0.47	0.40
12	0	4	1	U	1	1	1	1	U	U	U	U	U	1	U	1	U	0.47	0.73	0.12
13	1	−1	1	U	1	U	1	1	U	U	U	U	1	U	0	1	U	0.40	0.67	0.62
14	1	0	1	U	1	1	1	1	0	1	1	1	0	1	0	1	0	0.67	0.70	0.64
15	1	1	1	U	1	U	1	1	1	1	1	1	1	1	0	1	0	0.73	0.80	0.46
16	1	2	U	U	1	U	1	1	U	1	1	1	1	1	0	1	0	0.60	0.73	0.41
17	1	3	U	U	1	U	1	1	U	1	1	1	1	1	0	1	0	0.60	0.73	0.30
18	1	4	1	U	1	1	1	1	0	0	0	1	0	1	0	1	0	0.53	0.57	0.46
19	2	−1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0.13	0.13	1.08
20	2	0	1	U	U	U	1	1	U	1	1	1	1	1	0	1	0	0.60	0.73	0.98
21	2	1	1	U	U	U	1	1	U	1	1	1	1	1	0	1	0	0.60	0.73	0.86
22	2	2	1	U	U	U	1	1	U	U	U	U	U	U	U	1	U	0.27	0.63	0.68
23	2	3	1	U	U	U	1	1	U	1	1	1	1	1	0	1	0	0.60	0.73	0.47
24	2	4	U	U	1	U	U	1	0	0	0	0	0	1	0	1	0	0.27	0.40	0.59
25	3	−1	1	0	0	0	0	1	0	1	0	1	1	1	0	1	0	0.47	0.47	1.57
26	3	0	0	0	1	0	0	0	U	1	1	1	0	0	0	1	0	0.33	0.37	1.35
27	3	1	1	0	0	0	0	1	0	1	1	1	1	1	0	1	0	0.53	0.53	1.26
28	3	2	1	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0.33	0.33	1.04
29	3	3	1	0	0	0	0	1	0	1	1	1	1	1	0	1	0	0.53	0.53	0.65
30	3	4	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0.27	0.27	0.81
31	I	−1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0.07	0.07	3.17
32	I	0	0	U	U	0	U	0	U	U	U	1	0	0	0	1	0	0.13	0.33	2.48
33	I	1	1	0	U	0	0	U	0	0	0	1	0	1	0	1	0	0.27	0.33	1.44
34	I	2	U	0	U	0	0	U	U	U	U	U	U	U	U	1	U	0.07	0.43	1.18
35	I	3	U	0	U	0	0	U	U	0	0	1	0	1	0	1	0	0.20	0.33	0.80
36	I	4	U	0	U	0	0	U	0	0	0	0	0	0	0	1	0	0.07	0.17	1.02

[a] Logarithm (base 2) of the transversion–transition cost ratio. A value of 0 signifies a cost ratio of 2^0 or 1, where transitions and transversions cost the same. I = an infinite cost ratio (transition cost = 0; transversion parsimony).

[b] Logarithm (base 2) of the gap–change cost ratio (C_{max} denotes the maximum change cost, i.e., between transversions and transitions, the costlier).

[c] Ml = Mollusca; An = Annelida; Cl = Clitellata; At = Arthropoda (sensu Weygolt, 1986; Onychophora + Euarthropoda); On = Onychophora; Eu = Euarthropoda (sensu Weygolt, 1986); Ch = Chelicerata; Ec = Euchelicerata; Ac = Arachnida; Ar = Araneae; Mn = Mandibulata; Cr = Crustacea; Tr = Tracheata (Myriapoda + Hexapoda); My = Myriapoda; Hx = Hexapoda. 1 = monophyletic under those conditions; 0 = resolved nonmonophyly; U = lack of resolution potentially consistent with monophyly.

[d] Percent shared groups, calculated by summing up the 1 cells for the cost and dividing by the total number of groups examined (n = 15).

[e] Percent consistent groups, calculated as in PSG but includes the U entries as a value of 0.5.

[f] Mickevich–Farris extra-steps index ($\times 10^{-2}$).

DISCUSSION

Taxonomic and Character Congruence

The congruence surfaces for taxonomic and character measures show a great difference in relief. The PSG (taxonomic) index varies from a low of 0.07 to a high of 0.73, whereas the PCG values are somewhat higher (as expected), from 0.07 to 0.80 (Table 2). The Mickevich–Farris index varies from 0.0012 to 0.0317. Although the

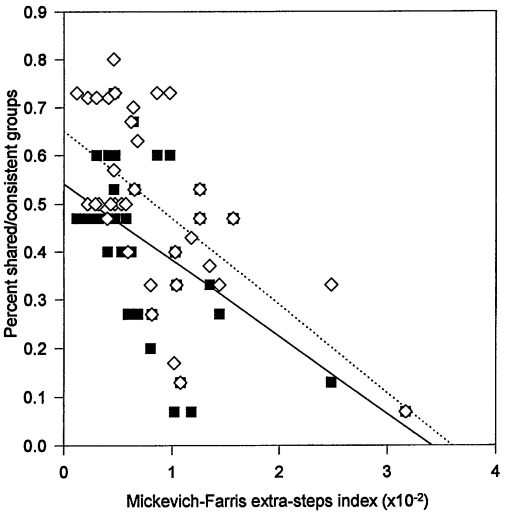

FIGURE 4. Regression of percent shared groups (PSG, solid line, ■) and percent consistent groups (PCG, dashed line, ◇) on the Mickevich–Farris extra-steps index ($\times 10^{-2}$).

taxonomic congruence has a greater absolute range, the character-based comparisons show a relative range of a factor (high/low value) of 26.4, whereas the PSG and PCG show less than half as much relief (10.4 and 11.4, respectively).

The relative maxima of the two measures are not completely concordant. Taxonomic congruence values show maxima at a gap–change cost ratio of 2:1, with a 2:1 transversion–transition cost ratio. The character congruence maximum occurs where transversions and transitions are equal, with gaps costing 16 times changes. This maximum is also in an area of taxonomic congruence (the second highest value for PCG). Part of this discordance between measures results from the placement of one taxon, the spider *Nephila*, that is extremely sensitive to analysis parameters (this instability may be due to the large number of unique character states observed in this taxon). In these areas of maximum character-based congruence, *Nephila* is placed not with other chelicerates but within the hexapods. This placement results in the necessary nonmonophyly of 7 of the 15 groups examined.

Although the measures are not entirely

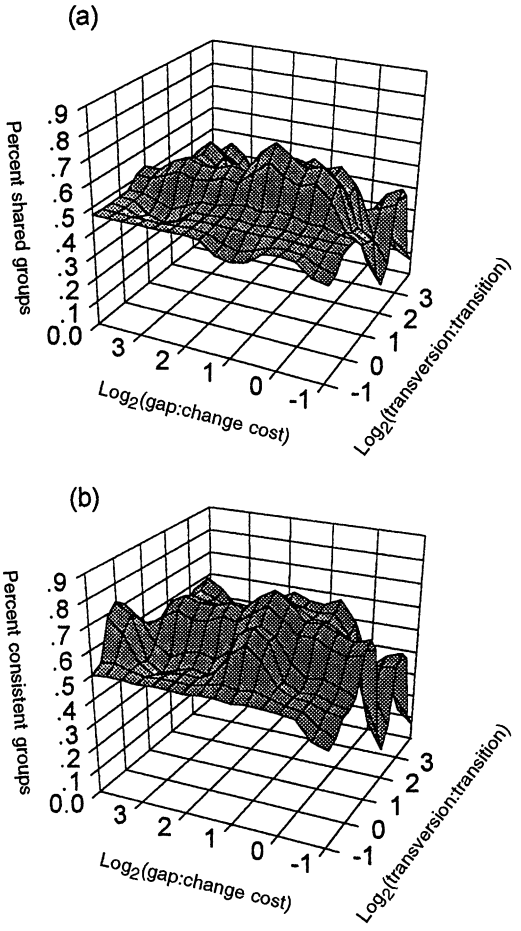

FIGURE 5. Plots of taxonomic congruence with respect to transversion–transition and gap–change cost ratios. The independent axes are incremented in logarithmic (base 2) units as in Figure 1. Higher values denote greater taxonomic congruence between data sets. (a) Percent shared groups. (b) Percent consistent groups.

in step, they are largely in agreement (minima are coincident but maxima differ). Additionally, unresolved cladograms can have complete character congruence because the imposition of characters that yield an additional unresolved bush requires no extra homoplasy.

One large area of agreement between these two measures is where/when the transversion–transition cost ratio is infinite (transitions have zero cost). Both the taxonomic and character incongruence is high

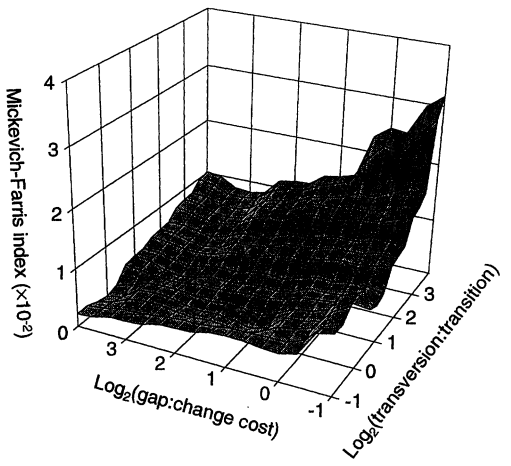

FIGURE 6. Plot of character congruence as assayed by the Mickevich–Farris extra-steps index. The index ($\times 10^{-2}$) is plotted with respect to transversion–transition and gap–change cost ratios. The independent axes are incremented in logarithmic (base 2) units as in Figure 1. Lower values denote greater character congruence between data sets.

here (Figs. 5, 6). Additionally, the total exclusion of transition information was most damaging to the resolution of groups with some of the most unresolved cladograms in the entire analysis space. Clearly, the exclusion of transition information is a bad course to take with these data.

Phylogenetic Analysis as a Decision Process—Areas of Monophyly

If the status of individual groups such as Chelicerata or Arthropoda is plotted in the two-dimensional parameter space (transversion–transition and gap–change cost ratios), areas of monophyly are produced (Fig. 7). The boundaries of these areas are the lines where the decision to accept or reject a hypothesis changes. The size and number of these areas give a measure of the generality and stability of the hypothesis of monophyly. If a high fraction of the total analysis space supports a group, the group is generally supported by the data because most combinations of analytical parameters will yield that clade, especially if the areas of support are contiguous. If, however, the areas in which the clade is supported are broken up and distributed over the space, this group (however general) would be unstable because small perturbations in analysis would lead to a new result. These two measurements provide means of comparing the robustness of different groups with respect to both their generality and stability. The eucheliceratas and arthropods (sensu Weygolt, 1986) are monophyletic in 31% and 36%, respectively, of the analysis space examined (Table 2). For the Eucheliceratа, the area supporting monophyly is contiguous. However, there are two areas in which the arthropods are supported as monophyletic. The disjoint nature of this pattern and its overall low level speak to the instability of this clade with respect to these data.

Phylogenetic analysis can be thought of as a decision-making process in which one is presented with two options: monophyly and nonmonophyly. The null hypothesis then would be that a particular group is nonmonophyletic. The type of analysis presented here allows the examination of the conditions that affect such a decision. Because the following discussion is based on taxa, taxonomic congruence criteria will be used for error minimization.

In any inference or decision-making problem, the combination of two types of error should be minimized: the erroneous rejection (Type I, α) or acceptance (Type II, β) of the null hypothesis. No decision-making procedure can minimize both simultaneously, so most inference procedures attempt to minimize some combination of the two. The minimization of Type I and Type II errors individually (but nontrivially) yields the extremes of maximally conservative (but low power) decisions on one side and maximally credulous (but powerful) decisions on the other.

In terms of phylogenetic analysis, the conservative approach would yield robust but less resolved cladograms than would the more aggressive (resolved) decisions. These aggressive decisions would yield the most resolved (hence informative) cladograms. The most robust cladogram implicit in these data would only contain (unfortunately) a single clade (Myriapoda, Fig.

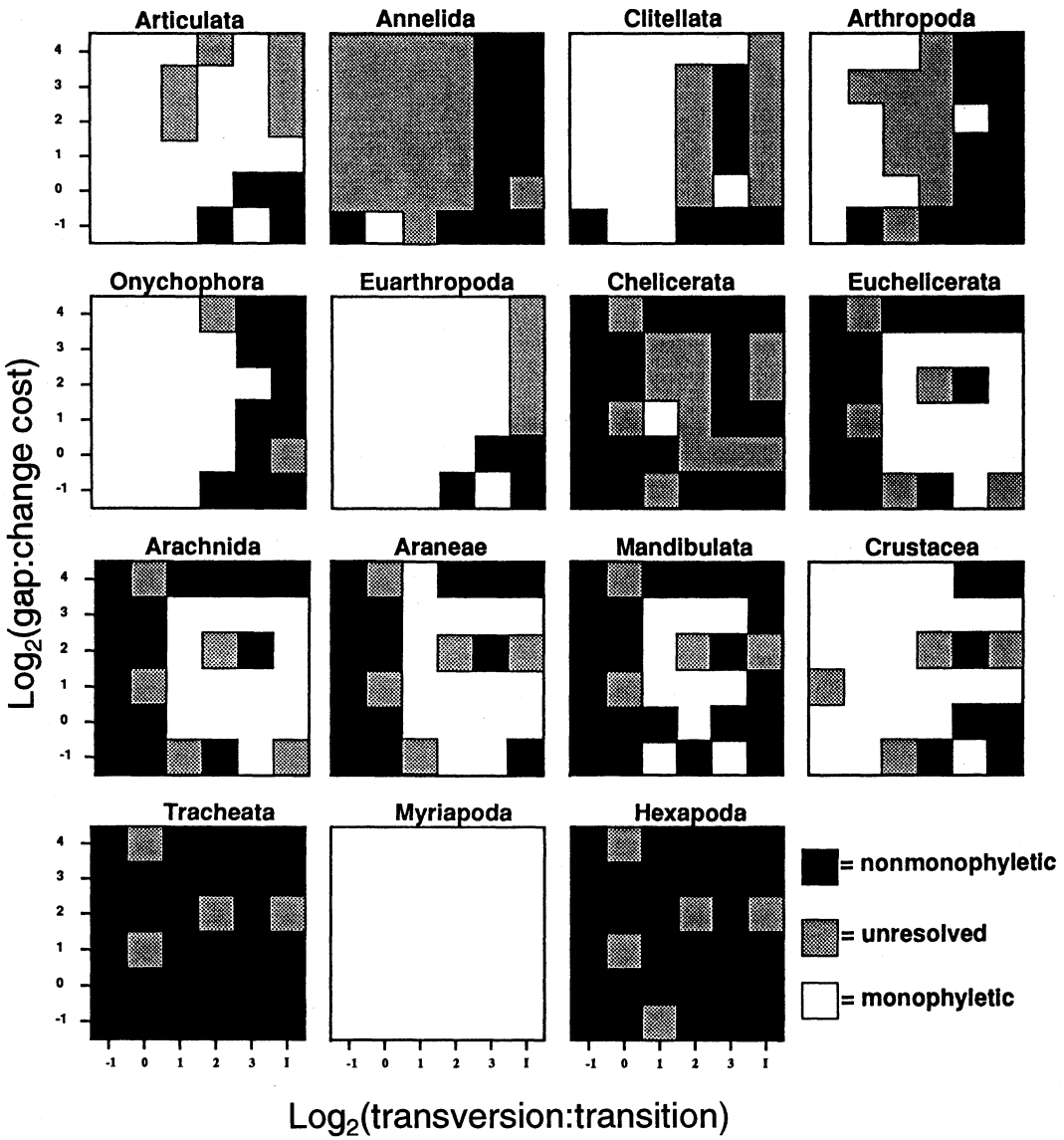

FIGURE 7. Gallery of analysis space plots for each of the 15 groups in Table 2 assayed for monophyly. ■ = positively nonmonophyletic (resolved and not monophyletic); ▩ = lacking resolution but potentially consistent with monophyly; □ = monophyletic. Axes are as in Figure 1.

8a). The myriapods appear together in 100% of the analyses performed. If we relax this criterion to 75% (shifting error emphasis from minimizing Type I to minimizing Type II), an additional component appears resolved (Euarthropoda). This process of progressive relaxation can be continued adding more groups at lower levels. Although this process contains the elements of robust analysis, it is in fact a poor decision-making process because extremely unlikely values of parameters and those that seem more reasonable are given equal force (i.e., yield more congruent results) in determining the disposition of groups. Additionally, the justification of

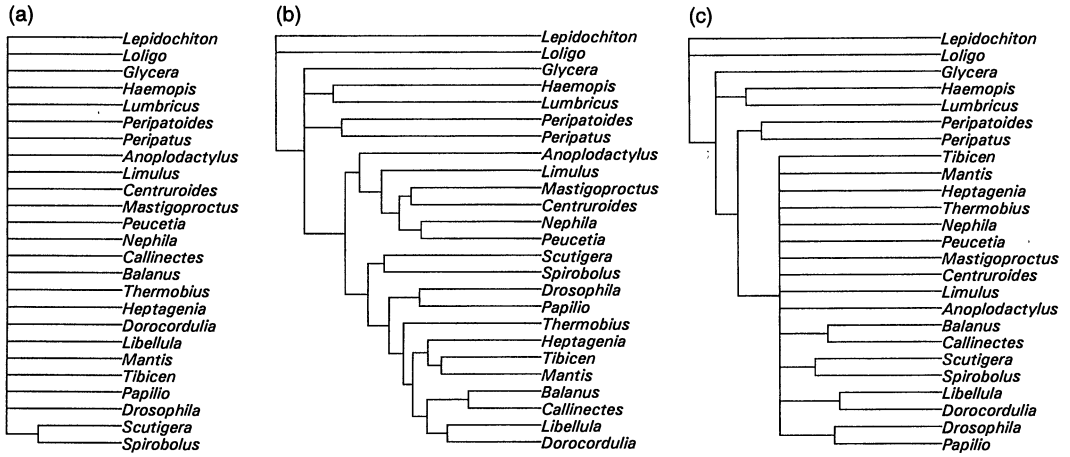

FIGURE 8. Cladograms of arthropods and related taxa. (a) Single component present in all analyses—Myriapoda. (b) Maximum taxonomic congruence (both PSG and PCG). (c) Maximum character congruence (minimum Mickevich–Farris index). The cladograms were created with Clados (Nixon, 1992).

any threshold value other than 100% is problematic. When a particular component is present in all the cladograms produced in the parameter space (i.e., resolved in a grand strict consensus of all generated cladograms), its acceptance seems well justified. The process of accepting progressively less frequently represented components leads to the "slippery slope" (A. Kluge, pers. comm.) phenomenon, where no objective stopping point can be determined and only personal credulity respected.

The more liberal approach of explicitly maximizing congruence will yield a greater number of resolved components that, although congruent, are less likely to be robust to variation in analysis parameters. The cladograms that are the best, or most congruent, present many resolved and congruent components (taxonomic congruence [Fig. 8b], character congruence [Fig. 8c]) as opposed to the lone Myriapoda yielded by more conservative analysis (Fig. 8a). The sets of parameters that yielded maximally congruent analyses also yielded a highly resolved cladogram when combined with morphological information in a total evidence (Kluge, 1989) framework (Fig. 9).

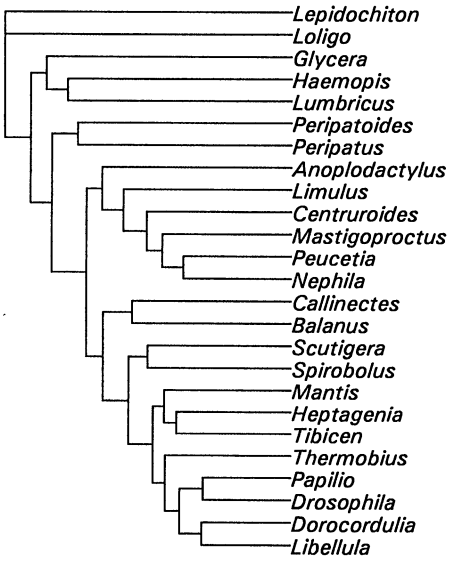

FIGURE 9. Cladogram derived from total evidence analysis of the combined morphological data of Wheeler et al. (1993) and the data derived from the analysis parameters that yielded the maximal taxonomic congruence (transversion–transition cost ratio = 2; gap–change cost ratio = 2). The total evidence cladogram contains Tracheata. This clade is not supported in any of the molecular analyses alone; the interaction of molecular and morphological data is required to support this group. The cladogram was created with Clados (Nixon, 1992).

CONCLUSIONS

Although the specifics of individual data sets will vary, the means of analyzing sequence data presented here allows the examination of the influence of unmeasurable parameters on phylogenetic analysis. Conservative and liberal hypotheses can be erected that bound the area between maximal (more specific) and minimal (more general) assumption, allowing reasonable risk assessment by trading resolution for generality. Through the explicit examination of the parameters of analysis (there are many more than the two examined here, e.g., codon position and bias, positional weighting) coupled with the use of congruence based optimality, assumption-specific unstable conclusions can be avoided.

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