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ON WEIGHTING AND CONGRUENCE

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Abstract — A priori differential weighting of molecular characters is a common methodological practice in molecular phylogenetics and evolution. This has been a largely subjective exercise with few criteria for deciding which characters to down-weight and how much to do so. A priori differential weighting is conducted to remove heterogeneity from the data sets and to improve the congruence among the informative, and usually more conservative characters. Herein, we test whether congruence is improved with a priori differential weighting by using the incongruence length difference test on a linked genetic data set consisting of 14 mammalian taxa and the 13 protein coding genes of the mitochondrial genome. Weighting by omitting the third codon position did not improve congruence with respect to the equally weighted data, while weighting transversions did improve the congruence between the 13 protein coding genes. Nonetheless, the most parsimonious tree found from transversion weighting did not differ from one using all of the data equally weighted.

Introduction

Numerous investigators have attempted to infer higher level mammalian phylogenies using the most conservative regions and substitutions of mtDNA. Genes which have been used for these problems include NADH dehydrogenase subunits 4 and 5 (Brown et al., 1982; Hayasaka et al., 1988; Cracraft and Helm-Bychowski, 1991), cytochrome b (Irwin et al., 1991; Irwin and Wilson, 1993; Irwin and Arnason, 1994), the displacement loop (Saccone et al., 1991), COII (Ruvolo et al., 1991; Disotell et al., 1992; Adkins and Honeycutt, 1991, 1994), and 12S and 16S rRNA (Thomas et al., 1989; Miyamoto and Boyle, 1989; Miyamoto et al., 1990; Kraus and Miyamoto, 1991; Mindell, 1991; Mindell et al., 1991; Allard et al., 1991; Allard and Honeycutt, 1992; Allard et al., 1992; Ammerman and Hillis, 1992; Knight and Mindell, 1993; Milinkovitch et al., 1993; Springer and Kirsch, 1993; Gemmell and Westerman, 1994).

Virtually all of these studies have involved a priori differential weighting of the molecular characters. Numerous procedures have been proposed for character weighting. For nucleotide sequence data, this is usually done either by a priori differentially weighting substitutions (e.g. transversions versus transitions), different positions within the molecule, such as codon positions or stems versus loops, or across both positions and substitutions. The amount of weight to be applied to the characters (a priori) has always been a rather subjective exercise, thus a wide range of weights have been suggested, with the most frequent of these being to drop one set of substitutions or positions by giving them a weight of zero (Adkins

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and Honeycutt, 1991, 1994; Allard et al., 1992). It is not uncommon to find transition to transversion differential weighting ratios of zero to one, one to one, one to two, one to five, and one to 10, often within a single phylogenetic study (e.g. Vrana et al., 1994). A separate strategy, and one which we will not discuss in detail is a posteriori differentially weighting (i.e. successive weighting; Farris, 1969) whereby characters are weighted based on the levels of homoplasy found with respect to a phylogeny. The concept of successive weighting (a posteriori) has a more defensible rationale (Carpenter, 1994), although the details of the method may be disputed (Goloboff, 1993).

More exotic strategies for weighting also have been proposed, based on linear and quadratic relationships to the frequency of the observed character change (Williams and Fitch, 1990; Fitch and Ye, 1991), providing additional choices of possible weighting strategies. To date, no objective methodologies have been proposed for choosing among this bewildering diversity of possible weights. One problem with his subjectivity is that differential weighting strategies will often produce different tree topologies. Thus, one is left with little justification for picking character weights and the final topology derived from it. For this reason the conventional practice has been for analyses to start with equal weighting of all characters and to proceed with differential weighting if the phylogenetic results are not congruent with other evidence (e.g. morphological hypotheses) or if there is evidence for saturation in the genetic data.

Our position is that no differential weighting can be justified without some intrinsic factor supporting the special nature of one set of characters over another. A possible example of an intrinsic factor was presented by Wheeler and Honeycutt (1988) when they showed that small nuclear ribosomal subunits show a strong co-evolutionary response to substitutions in the double stranded stem regions. For this reason they suggested that down-weighting of these stem regions was justified for phylogenetic analysis. As different ribosomal genes show different levels of co-evolutionary response, even this example may only be particular, not representing a general approach, in regards to these effects on the phylogeny.

Frequently greater weights are given to the more conservative characters under the general reasoning that the phylogenetic pattern for these will not be confounded by the effects of multiple substitution at a site. Any substitutions occurring on top of a previous one might produce a parallelism or reversal and by down-weighting these more variable sites phylogeneticists believe that they are removing heterogeneity, often referred to as noise, from their phylogenetic analyses. It is our contention that multiple substitutions can provide additional phylogenetic information and further resolution to the final topology. Nonetheless, it is commonplace for molecular systematists to use some pairwise method to assess levels of multiple substitution a priori (e.g. saturation curves) in the absence of a phylogenetic perspective, and then remove those characters found to be affected (Mindell and Honeycutt, 1990; Irwin et al., 1991; Brown et al., 1992; Miyamoto et al., 1994). Most frequently, either transitions or third codon positions are removed or down-weighted in an analysis (see reviews by Moritz et al., 1987; Simon et al., 1990; Swofford and Olsen, 1990; Hillis et al., 1993).

It is generally considered that the bias from the characters affected by multiple substitutions will lead to the more conservative characters being swamped out by the more variable (Bull et al., 1993), and often more numerous ones. Thus,

differential weighting is an attempt by investigators to remove heterogeneity from their data sets and to increase the congruence among characters within a data set. This common strategy for phylogeny reconstruction has rarely been rigorously tested to determine if heterogeneity is in fact removed and congruence improved through weighting.

Available methods for measuring the agreement within and between data sets include measures of consistency (e.g. CI, RI) and congruence (Mickevich and Farris, 1981; Miyamoto et al., 1994). While it is generally recognized that differential weighting does not always produce greater levels of consistency for the same character matrix, these measures may not be appropriate for comparisons across different analyses (see comments in Farris, 1989; Goloboff, 1993). Recently, measures based on the incongruence length difference test (ILD) have been used in sequence alignment (Wheeler, 1995) and statistical testing between data set congruence (Farris et al., 1994). The ILD test is implemented in the computer programs Arn (Farris, 1991), DADA (Nixon, 1994), and Arnie (Sidall, 1995).

The basic idea is that if one uses parsimony to assess how well characters fit a particular hypothesis, then one will choose the best tree according to the one that provides the simplest explanation of the data (i.e. the one that requires the fewest ad hoc hypotheses). If the characters within the data set agree (i.e. are congruent) with one another then the result will be a strongly supported hypotheses with the alternative solutions being poorly supported and requiring additional ad hoc hypotheses to explain the data. When the evidence is weak or ambiguous then an alternative hypothesis will require fewer additional ad hoc hypotheses to explain the results. One can extend this argument from within data sets to between data sets by comparing how well one data set fits the results of another data set (Miyamoto et al., 1994). Thus, weak or ambiguous results are congruent more often with other data sets. This effect of congruence has confounded strategies for determining whether one has congruence because there are overlapping signals present in the data sets or whether one or both of the data sets is ambiguous (Mickevich and Farris, 1981). When the results of phylogenetic analyses (e.g. tree topologies) are compared this is known as taxonomic congruence; and when the data themselves are compared this is known as character congruence (Kluge, 1989). The incongruence length difference test is a statistical method of character congruence. It uses the incongruence length difference to estimate incongruence by resampling a data matrix from the pool of characters from the combined original matrices. If the two, or more, original data sets are incongruent then the added tree lengths, for the most parsimonious solutions, from the resampled matrices will be greater than the added tree lengths from the original matrices (Fig. 1). The larger tree lengths are due to the additional character incongruence (i.e. character disagreement over phylogenetic pattern) created when the data are combined in the pooled sample (Farris et al., 1994).

Herein, we test whether weighting strategies improve the congruence between data sets by estimating the incongruence length difference for equally weighted data versus differentially weighted data. If weighting is removing heterogeneity from molecular data sets then we predict that these differentially weighted matrices should show improved levels of congruence as well. If no differences are measured between the alternative weighting strategies, then this suggests that there has been no removal of the heterogeneity in these data sets. If this were

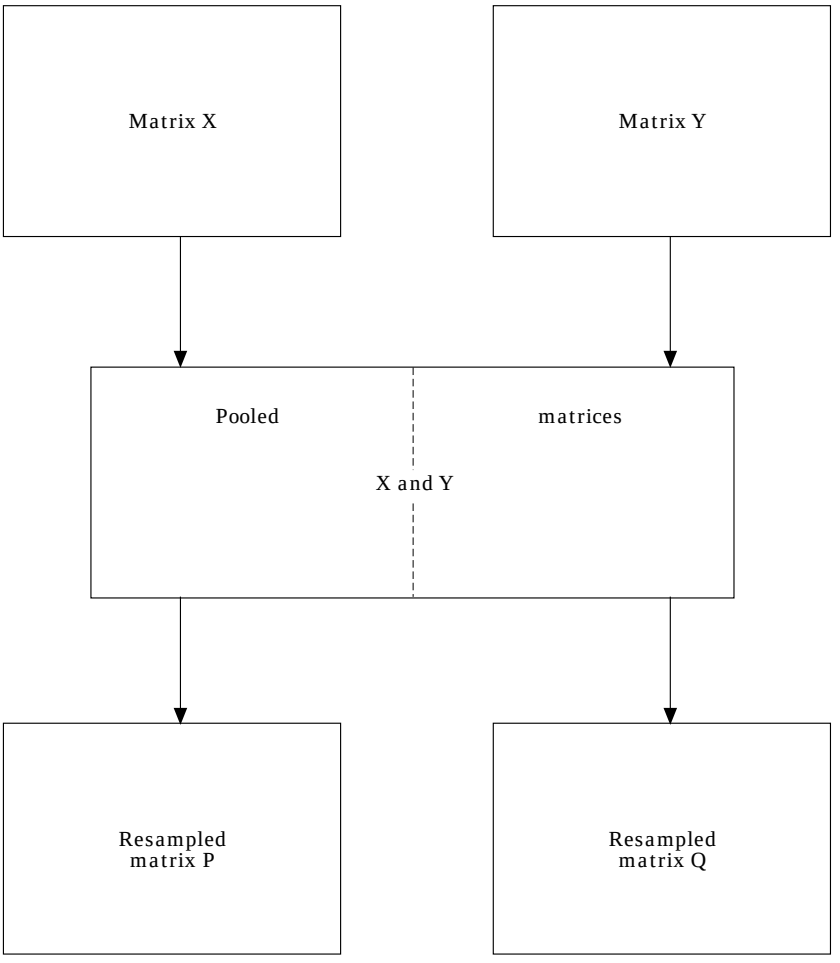

Fig. 1. Schematic of the incongruence length differences test (Farris et al., 1994). The data matrices are incongruent when the sum of the tree lengths (L) of the original matrices is shorter than that for the resampled matrices, $(L_X+L_Y)<(L_P+L_Q)$. Significant incongruence indicates rejection of the null hypothesis of congruence among the partitions.

observed then we would recommend that all characters be included and equally weighted in the analysis, as there is no demonstration that the presumed heterogeneity in the original characters had been corrected. Without a clear reason for differentially weighting characters it would seem better to leave the data as equally weighted, otherwise one indulges in ad hoc hypothesis building.

Genetic Linkage and Congruence

The use of linked mitochondrial sequences holds promise for improving our understanding of character weighting, reliability, and congruence. The general

theory for using linked mtDNA sequences is that each gene contained in the linked region has shared the same gene phylogeny for the duration over which the linkage has been maintained, presumably due to the lack of recombination (Avise, 1991; Miyamoto et al., 1994). We will use the incongruence length difference, to test whether weighting methods are able to improve the congruence among data sets pertaining to ordinal relationships among the Mammalia. Congruence among data sets could be directly affected by the amount of linkage between the various genes analysed. Numerous genetic processes such as gene duplication, lateral transfer of genetic material, ancestral polymorphisms, gene conversion, and other sources of paralogy, xenology and recombination can sometimes confound phylogenetics, with the possibility of different histories for unlinked genes co-existing within a single taxon. Most of the effects from these genetic processes can be eliminated by taking advantage of the natural properties of complete mitochondrial genomes. In particular many of these processes are negated by the fact that all of the genes in mitochondrial DNA are genetically linked. Thus, comparisons across mitochondrial genomes should show higher levels of congruence than unlinked genes due to their shared genetic history (Miyamoto et al., 1994). Linked DNA analysis cannot be performed on the majority of nuclear genes, which undergo recombination. The existence of conflicting gene trees for mammalian relationships is well documented (Miyamoto and Goodman, 1986; Ammerman and Hillis, 1992; Honeycutt and Adkins, 1993; Novacek, 1994) and this plethora of tree topologies has complicated the determination of the phylogeny of Mammalia. If multiple trees are found with linked mtDNA sequences, this cannot be due to different gene histories but must be due to other factors such as levels of homoplasy. In this study we will examine three alternative weighting strategies in an attempt to uncover methods which improve the congruence between data sets and assist in our efforts to find more reliable characters to support the most divergent mammalian lineages.

Linkage is predicated on no recombination, nonetheless, the absence of recombination is not a certainty. Recombination is known to occur in yeast, and rearrangements in gene order have been reported in numerous organisms (Avise, 1991; DesJardin and Morais, 1990; Paabo et al., 1991; Seutin et al., 1994). The fact that gene order does not remain constant for all vertebrate mtDNA genomes means that some rearrangements have occurred (but see Quinn and Wilson, 1993). Even within the Mammalia, rearrangements have been discovered; notably, the opossums have several rearrangements with respect to the Eutheria (Päabo et al., 1991; Janke et al., 1994). Nonetheless, no rearrangements are evident within the Eutheria and thus this is a good example of linkage which should eliminate most of the incongruence due to gene tree effects.

Mitochondria are directly affected by ancestral polymorphisms and allelic sorting, both at the population level and within individuals (e.g. heteroplasmy). While not fully alleviating all of the gene tree effects, most of the mitochondrial genomes analysed here were sequenced from single individuals with only a few clones making up the entire sequence. To eliminate fully the possible effects of ancestral polymorphisms and allelic sorting, genes from single clones must be examined. However, even these precautions will not assure one of determining the “true” species tree rather than a mitochondrial gene tree, thus we make no claims concerning the truth about the resulting phylogeny.

Methods and Materials

In this analysis of linked mitochondrial genes the available complete mammalian mtDNA genomes were obtained from Genbank (Entrez version 17.0) for 14 mammalian species representing seven orders (Table 1). Each of the 13 protein coding genes were aligned with the assistance of the amino acid translations, after which the nucleotide sequences were subjected to parsimony analysis (Farris, 1988; Swofford, 1990). Three weighting treatments were followed for each separate gene, the first based on equal weighting of all informative characters, a second whereby the third codon position was down-weighted to zero and the other informative positions were equally weighted, and a third weighting all transversions equally and omitting the transitions. This latter differential weighting strategy was accomplished by reducing the original data to a two state character matrix with all bases coded as either a purine or a pyrimidine. This is in contrast to transversion weighting via a step matrix which does not treat all purines, or all pyrimidines, as equivalent (Edwards et al., 1991). All genes were analysed both separately and combined in a simultaneous analysis (Kluge, 1989; Nixon and Carpenter, 1996).

If multiple most parsimonious trees (MPT) were found then successive approximation weighting was applied (Farris, 1969; Carpenter, 1988). If this method did not reduce the trees to a single solution then a strict consensus tree was constructed from the remaining topologies. An opossum (order Marsupialia, *Didelphis virginiana*) sequence was used as an outgroup in all analyses to root the tree. The opossum is considerably more divergent than the eutherians compared in our analyses. Thus, one would expect significantly more homoplasy to be added to the analysis from this taxon alone. This outgroup is included for two reasons: (1) it is an appropriate outgroup for the Eutheria based on morphological evidence; and (2) because the ingroup to outgroup comparisons are most likely to benefit from differential weighting if heterogeneity is present and can be removed (Allard et al., 1996).

Table 1
Mammal orders used in our analyses, with GenBank accession numbers, species, common names and references provided for each sample

Order	OTU	GenBank #	Species	Common name
Artiodactyla[1]	BTA	V00654	*Bos taurus*	cattle
Perrisodactyla[2]	ECA	X79547	*Equus caballus*	horse
Cetacea[3]	BPH	X61145	*Balaenoptera physalus*	fin whale
	BMU	X72204	*Balaenoptera musculus*	blue whale
Primates[4]	HSA	D38112	*Homo sapiens*	man
	PPY	D38115	*Pongo pygmaeus*	orangutan
	PPA	D38116	*Pan paniscus*	pygmy chimp
	PTR	D38113	*Pan troglodytes*	common chimp
	GGO	D38114	*Gorilla gorilla*	gorilla
Rodentia[5]	MMU	V00711	*Mus musculus*	mouse
	RNO	X14848	*Rattus norvegicus*	rat
Carnivora[6]	PVI	X63726	*Phoca vitulina*	harbor seal
	HGR	X72004	*Halichoerus grypus*	grey seal
Marsupialia[7]	DVI	Z29573	*Didelphis virginiana*	opossum

Note—References are as follows: 1, Anderson et al., 1982; 2, Xu and Árnason, 1994; 3, Árnason et al., 1991; Árnason and Gullberg, 1993; 4, Horai et al., 1995; 5, Bibb et al., 1981; Gadaleta et al., 1989; 6, Árnason and Johnsson, 1992; Árnason et al., 1993; 7, Janke et al., 1994.

Congruence between data sets was estimated using the incongruence length difference test as implemented in the program Arn (Farris et al., 1994). The 13 protein coding genes were combined as 13 partitions of the data with 1,000 resamplings examined for each analysis. The incongruence length difference test was calculated for three separate weighting strategies in an attempt to determine whether omitting transitions or third codon positions from data matrices could improve the congruence among the data sets.

Results

Alignments for all of these genes are available from the EMBL server by e-mailing the request (GET ALIGN:DS27019.DAT) to NetServ@EBI.AC.UK. Parsimony solutions for each of the genes, run both separately and simultaneously, are listed in Table 2 and Fig. 2. Considerable variation in tree topology is observed for each of the separate genes (Fig. 2A to CC), while simultaneous analysis including all of the genes produced two trees, one of which was identical under two of the weighting strategies (Fig. 2A) and a second which was found when third positions were omitted (Fig. 2N).

Eleven of the genes produced multiple equally parsimonious solutions, depending on the weighting strategy employed. For these data sets we could not reach a single solution for three of the matrices even after applying successive weighting. For these three analyses, strict consensus trees were constructed from the equally parsimonious solutions. In every case, the polytomies produced in the strict consensus trees involved members of the order Primates (Fig. 2 O, T, and U).

Of the 29 topologies produced from the 42 analyses (i.e. 13 separate genes for three weighting strategies, and three simultaneous analyses), six topologies were found by more than one analysis (Table 2 and Fig. 2). Topologies K and R were found most frequently (four times) both when analysing various NADH subunits and the cytochrome b gene. Topology A was obtained three times and topologies H, N, and S were found twice each. Topology N was found once with the simultaneous analysis for third codon position omitted and once for transversion parsimony of NADH1. The simultaneous analysis for all characters weighted equally, transversions weighted differentially, and transversion parsimony of NADH4, support topology A as most parsimonious. The 13 separate genes produced 12 topologies (Fig. 2 E, F, H, I, J, K, L, P, Q, R, S, V) when the data were equally weighted, 12 topologies (Fig. 2 A, B, C, D, G, K, M, N, O, R, T, U) when transversions were weighted greater than transitions, and 11 topologies (Fig. 2 H, R, S, V, W, X, Y, Z, AA, BB, CC) when the third codon position was omitted. Differential weighting did not improve the agreement between MPT for these 13 linked mitochondrial genes. The strict consensus trees of the possible single gene topologies, for each of these weighting strategies, supported one resolved node each (i.e. PPA, PTR; MMU, RNO; and MMU, RNO; respectively, for equal weights, transversions weighting, and codon weighting).

The incongruence length difference test was calculated for each of the three separate weighting procedures (equal weighting, third codon position omitted, and transversion weighting) for the simultaneous analyses of all of the genes. For equal weighting, the data matrices were significantly incongruent (alpha=0.001,

observed ILD=111, randomized range=0–111) and this was also found when third codon positions were omitted (alpha=0.001, observed ILD=70, randomized range= 0–70), thus rejecting the null hypothesis of congruence. Transversion weighting did improve the congruence between the various mtDNA genes (alpha=0.495, observed ILD=4, randomized range=0–71), thus, between data set congruence was not rejected. Therefore, if one accepts the primacy of character congruence then the topology obtained from transversion weighting would be accepted as the best explanation of the available congruent mitochondrial sequences (Fig. 2A). Our inability to demonstrate the utility of omitting third codon positions left us with no justification for doing so.

Table 2(a)

Tree information for the most parsimonious solution(s) for each of the genetic data matrices. Analyses were conducted using all substitutions, transversions only (via the EQUATE command), and omitting the third codon position for each of the 13 protein coding genes of the mitochondria. Topologies refer to those in Fig. 2. Abbreviations include: MPT, most parsimonious tree(s); SA, successive approximation weighting: TL, tree length. Informative characters refers to the number of informative characters in the data matrix as implemented in PAUP

All substitutions

	Topology	TL	# of MPT	SA analysis iterations: TL	SA topology	Informative characters
Combined	A	16 518	1			5270
ATPASE6		338	2	1: 2 906	S	318
ATPASE8		329	4	1: 1 563	E	125
CO1	F	1 876	1			564
CO2	H	868	1			290
CO3	I	958	1			291
CYTB		1 469	3	1: 4 535	J	485
NADH1		1 258	2	1: 3 889	K	422
NADH2	K	1 814	1			580
NADH3	L	518	1			169
NADH4	P	2 028	1			654
NADH4L		417	8	1: 1 778	Q	139
NADH5	R	2 925	1			931
NADH6	V	859	1			302

Table 2(b)

Transversions only

	Topology	TL	# of MPT	SA analysis iterations, TL	SA topology	Informative characters
Combined	A	6470	1			3146
ATPASE6		90	3	1: 1 053	B	171
ATPASE8		135	5	2: 507	D	74
CO1		645	2	1: 2 183	G	298
CO2	C	294	1			150
CO3	M	336	1			158
CYTB	K	569	1			275
NADH1		494	2	1: 1 802	N	241
NADH2	K	814	1			401
NADH3		194	4	3: 69 624	O, strict	99
NADH4	A	803	1			398
NADH4L		155	2	3: 58 271	T, strict	82
NADH5		1213	2	1: 3 827	R	598
NADH6		349	3	3: 152 794	U, strict	201

The transversion weighted, simultaneous analysis, MPT (Fig. 2A) is not fully congruent taxonomically with an earlier mtDNA parsimony analysis (Honeycutt and Adkins, 1993) which was made up of a subset of these sequences and a more divergent outgroup (namely Aves and Amphibia). Since this previous analysis, over twice as many mammals and a marsupial outgroup have been sequenced. Our result differed from the Honeycutt and Adkins (1993) tree, in that we found Artiodactyla and Cetacea grouping rather than Artiodactyla and Carnivora. It is interesting to note that the Rodentia are placed as one of the earliest diverging extant lineages. This molecular hypothesis is fully resolved, including relationships for the orders Carnivora, Primates, and Rodentia (Fig. 2A). Within the Primates, the great ape trichotomy is resolved in favor of human and chimp being most closely related. The common and pygmy chimps are each others' sister group, with orang-utan as the most distantly related of the great apes examined. These results for the Primates are the same as those found by Horai et al. (1995).

Discussion

Our initial premise is that all of the genes from mitochondrial DNA should show the same tree, based on the one history of the linked elements. The fact that few of these genes analysed alone actually produce the same phylogeny suggests that other factors are affecting the phylogenetic outcome, such as not enough information for robust hypotheses, or that there is disagreement in the signal present in the various genes as to what the history was (see Cao et al., 1994; Cummings et al., 1995). We are able to measure this by the incongruence length difference test (Farris et al., 1994) which shows significant incongruence between the 13 linked mitochondrial genes.

Presumably there is heterogeneity in some of these genes that is causing the incongruence between the data sets. Exactly where such confounding characters lie has been at the heart of much of the current research in molecular systematics and evolution, though previous authors have suspected that the third codon position is a separate process partition from the first and second positions

Table 2(c)
Codon position 3 omitted

	Topology	TL	# of MPT	SA analysis iterations, TL	SA topology	Informative characters
Combined	N	5 158	1			2 233
ATPASE6		338	2	1: 2 906	S	133
ATPASE8	W	177	1			74
CO1		249	2	1: 4 843	X	115
CO2	H	188	1			102
CO3		169	7	1: 2 533	Y	74
CYTB		423	2	1: 4 566	V	180
NADH1		381	3	1: 4 116	Z	162
NADH2	AA	719	1			280
NADH3	BB	192	1			75
NADH4		653	2	1: 6 489	R	270
NADH4L	CC	135	1			59
NADH5		1 062	3	1: 9 108	R	413
NADH6		339	4	1: 3 495	V	163

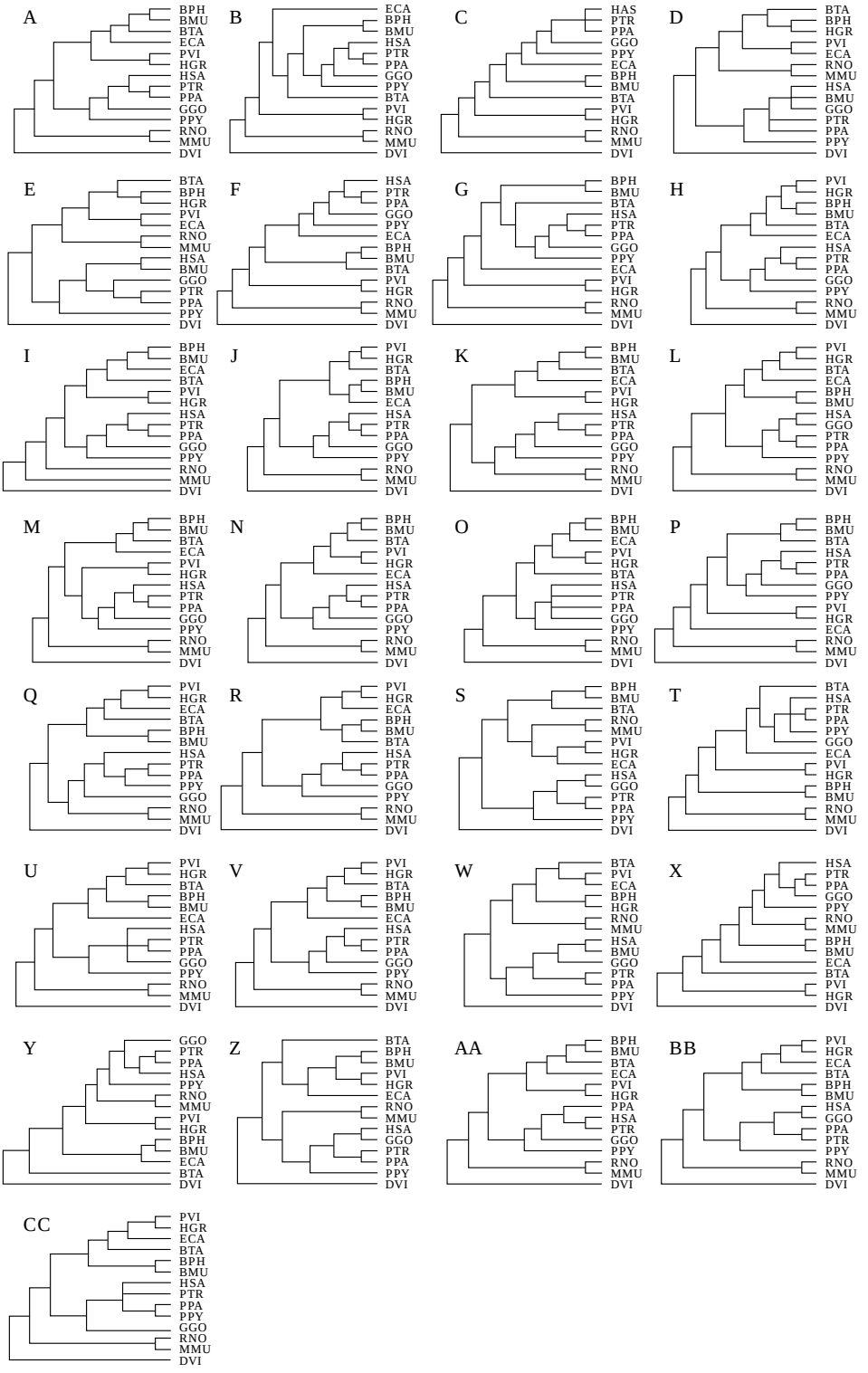

Fig. 2. Most parsimonious solutions determined from mitochondrial genes analysed both separately and simultaneously. Letters correspond to those listed in Table 2.

(e.g. Bull et al., 1993). It is generally believed that one can improve the phylogenetic signal (e.g. remove some of the heterogeneity) by differentially weighting the more conservative characters, especially for the most divergent taxonomic comparisons. Thus, it was surprising that the common strategy for down-weighting the third codon position did not produce congruence among these mtDNA genes. Presumably there are other heterogeneities in this data as well. It is heartening to know that we can improve congruence some of the time, as with our transversion parsimony analysis. Certainly we must be able to measure in some way whether we are actually removing heterogeneity rather than just assuming that this follows from our differential weighting. This is especially important as there are many strategies for weighting with each different treatment having the potential for providing an alternative phylogenetic hypothesis. Without a clear way to choose among these methods we are left with no justification for weighting other than ad hoc hypotheses to rationalize our treatments and topologies.

We have mainly discussed the effects of differential weighting on congruence, although other investigators have blamed unstable tree topologies and odd results on taxonomic sampling within the Mammalia (Stanhope et al., 1992; Allard et al., 1996). One could also use the incongruence length difference test for determining whether removing taxa improves the congruence between data matrices. If divergent outgroup or ingroup taxa are hypothesized as the cause for increasing incongruence, then the omission of these presumably problematic taxa should improve congruence among the data sets as well. We do not promote the use of this test for determining whether one should keep matrices separate, rather, this test is a means of determining whether a particular weighting strategy actually removes heterogeneity from a data set in comparison to another. Whether one should combine data sets is a separate issue and is of much concern in the current systematic debate (Kluge, 1989; Swofford, 1991; Bull et al., 1993; Miyamoto and Fitch, 1995; Nixon and Carpenter, 1996). Bull et al. (1993) decried the lack of a test to demonstrate heterogeneity within and between data sets, but the incongruence length difference test is such a method (Farris, 1991). Bull et al. (1993) argued that when heterogeneity (or incongruence) was found, the data sets should be treated as separate process partitions, with heterogeneity indicating either model failure or different histories.

However, we believe that it is better to include all available characters, and all matrices, in a single analysis of initially equally weighted characters, regardless of the level of incongruence observed between data sets (Nixon and Carpenter, 1996). Congruence is not the only goal of phylogenetic reconstruction and thus congruence should not be pursued over all others (Farris, 1989, p. 418). Rather, a search for completely congruent data should be considered another red herring, similar in futility to attempting to determine whether data are consistent or inconsistent (Allard, 1994). To demonstrate this we will take a closer look at several aspects of our results. While the transversion weighted data increased character congruence according to the incongruence length difference test, among the 13 gene partitions, results from the separate analyses did not improve agreement among the topologies, “taxonomic congruence”. The strict consensus trees from transversion weighted parsimony analyses [Table 2(b)] produced only one resolved node. This was also the case for the incongruent, equally weighted and third position omitted analyses. Thus, character congruence among data matrices,

as measured by the incongruence length difference, is not the same as topological congruence (also known as taxonomic congruence or topological correspondence; Kluge, 1989; Nixon and Carpenter, 1995). Furthermore, improving character congruence should not be confused with lack of resolution (i.e. ambiguity, see Mickevich and Farris, 1981).

Similarly, when examining the results of our simultaneous analyses, we find that improving the congruence in our transversion weighted analysis gave us the same tree as our incongruent equally weighted parsimony analysis. This suggests that while we may have improved character congruence and removed heterogeneity from our original combined matrix, this bias was insufficient to modify our phylogenetic conclusions. Thus, the concerns arising from Bull et al.'s (1993) simulated analyses, with regard to combining heterogeneous data, are not born out by these "real" data. The question arises, why bother with constructing a completely homogeneous data set? Similarly, why bother with a priori differential weighting of characters if we either cannot justify it or it does not affect our results? We predict that these results will continue to be observed as larger amounts of evidence are compiled by systematists. That is, in many cases it will no longer be necessary to massage the molecular data to improve taxonomic congruence and remove heterogeneity. Analysing all of the data, equally weighted, may even generally provide us with the same solution. For that matter, the final topology should not sway our decision on whether to use all of the data or not. Logical reasoning would suggest that simultaneous analysis is the appropriate action (Nixon and Carpenter, 1996); in this case we have empirical evidence which despite "heterogeneity" provides us with an example that this is so.

This test of character congruence will provide us with new ways to explore congruence both within and between data sets, using differential weighting and sub-sampling of the matrices, including omission of both characters and taxa. We have shown, for this mammalian data set, that congruence was rejected when all of the data were equally weighted. Nonetheless, this incongruence did not overwhelm the phylogenetic results found with transversions, a congruent secondary signal. We view the current trend of conducting multiple, unfounded weighting schemes as an outcome of insufficient taxonomic sampling and character information. As more characters per taxon are collected and analysed using simultaneous analysis, many of the current phylogenetic conflicts will presumably lessen, with the most appropriate a priori treatment being that of equal weighting of all data for most comparisons.

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