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Failure of the ILD to Determine Data Combinability for Slow Loris Phylogeny

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Abstract.—Tests for incongruence as an indicator of among-data partition conflict have played an important role in conditional data combination. When such tests reveal significant incongruence, this has been interpreted as a rationale for not combining data into a single phylogenetic analysis. In this study of lorisiform phylogeny, we use the incongruence length difference (ILD) test to assess conflict among three independent data sets. A large morphological data set and two unlinked molecular data sets—the mitochondrial cytochrome *b* gene and the nuclear interphotoreceptor retinoid binding protein (exon 1)—are analyzed with various optimality criteria and weighting mechanisms to determine the phylogenetic relationships among slow lorises (Primates, Loridae). When analyzed separately, the morphological data show impressive statistical support for a monophyletic Loridae. Both molecular data sets resolve the Loridae as paraphyletic, though with different branching orders depending on the optimality criterion or character weighting used. When the three data partitions are analyzed in various combinations, an inverse relationship between congruence and phylogenetic accuracy is observed. Nearly all combined analyses that recover monophyly indicate strong data partition incongruence ($P = 0.00005$ in the most extreme case), whereas all analyses that recover paraphyly indicate lack of significant incongruence. Numerous lines of evidence verify that monophyly is the accurate phylogenetic result. Therefore, this study contributes to a growing body of information affirming that measures of incongruence should not be used as indicators of data set combinability. [Conditional data combination; galagos; incongruence length difference; lorises; molecules and morphology; partition homogeneity test.]

The slow lorises (family Loridae; Gray, 1821) make up the most securely diagnosed clade in the entire primate radiation, both extant and extinct. These animals are distinguished from other primates by a spectacular array of behavioral, morphological, physiological, and ecological characteristics. A partial list of these characteristics includes fewer caudal vertebrae, more numerous thoracic vertebrae, transpedicular foramina of the thoracic vertebrae, shortened second digit of the hands and feet, retia mirabilia of the proximal limb vessels, large humeral and femoral articulations, highly mobile ankles and wrists, frontated and upwardly rotated orbits, specialized scent glands, slow methodical locomotion, digestive specializations for the consumption of toxic prey, and reduced basal metabolic rate (Charles-Dominique, 1977; Whittow et al., 1977; Muller et al., 1985; Rasmussen, 1986; Ikeda et al., 1988; Rasmussen and Izard, 1988; Yoder, 1994; Alterman, 1995; Runestad, 1997; Rasmussen and Nekaris, 1998). It is therefore surprising that the four genera that share these putative synapomorphies—*Arctocebus, Perodicticus, Nycticebus*, and *Loris*—are not also linked by genetic data. In fact, genetic studies have nearly uniformly failed to resolve the lorid clade. The only exception has been karyological studies, which have placed three of the four genera in a single clade (the fourth genus *Arctocebus* was not included in these studies) (Rumpler et al., 1987; Dutrillaux, 1988; Dutrillaux and Rumpler, 1995).

The slow lorises are currently found on two continents, Africa and Asia, and can be described as having one of two general body types, robust or slender. Africa possesses both a robust (*Perodicticus*) and a slender (*Arctocebus*) form, as does Asia (*Nycticebus* and *Loris*, respectively). Primate classifications have tended to emphasize either biogeography or morphology in dividing the lorids into subgroups. Those that emphasize geography therefore group *Perodicticus* with *Arctocebus* and *Nycticebus* with

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Loris (Groves, 1971), whereas those that emphasize general morphology group *Perodicticus* with *Nycticebus* and *Arctocebus* with *Loris* (Hill, 1947; Schwartz, 1992). One study concluded that the African and Asian lorisiforms form two distinct clades and that the elaborate morphological and behavioral similarities result from convergent evolution in the slow lorises (Groves, 1971). Several genetic studies support the hypothesis that the Loridae is diphyletic (Sarich and Cronin, 1976; Porter et al., 1997; Goodman et al., 1998), though only one, an immunodiffusion study, has suggested that the African slow lorises are more closely related to the African bushbabies (family Galagonidae) than to other slow lorises (Dene et al., 1976), which is in agreement with Grove's (1971) hypothesis. Although slow lorises and galagos are known to form a monophyletic suborder Lorisiformes (Charles-Dominique and Martin, 1970; Szalay and Katz, 1973; Rasmussen and Nekaris, 1998), the idea that the African slow lorises are related to galagos to the exclusion of Asian slow lorises is somewhat unorthodox. Goodman et al. (1998) have proposed an intermediate scheme in which African lorises, Asian lorises, and African bushbabies are each placed in their own subfamily (Perodictinae, Lorinae, and Galagoninae, respectively).

To investigate these phylogenetic discrepancies, we examined a mitochondrial gene known to be informative for strepsirrhine phylogeny (Yoder et al., 1996) and a nuclear gene known to be informative at a variety of phylogenetic levels within the Mammalia (Stanhope et al., 1992, 1996). These two independent molecular data sets were analyzed with phylogenetic methods to investigate their support for the presumed slow loris clade. DNA sequence data were generated and analyzed for all lorisiform taxa for which tissues were available. Given the difficulty in acquiring tissue for the genus *Arctocebus*, our taxon sample includes only three of the four slow loris genera (*Perodicticus*, *Loris*, and *Nycticebus*). We have also analyzed a large morphological data set to investigate its support for the slow loris clade. The morphological and two molecular data sets were analyzed separately and in a variety of combinations, with both equal and differential weighting. We used the incongruence length differential (ILD) test (Mickevich and Farris, 1981; Farris et al., 1994, 1995) to investigate the degree of heterogeneity among data sets. Results of the ILD tests were then compared with tree topology and bootstrap support relative to resolution of the slow loris clade.

What is the ILD Test?

Several studies have suggested that data sets should not be combined for phylogenetic analysis unless the data partitions are demonstrably not heterogeneous or otherwise in conflict (Bull et al., 1993; de Queiroz, 1993; Huelsenbeck et al., 1996). Although numerous methods have been developed for exploring the degree to which data sets conflict with one another (Templeton, 1983; Rodrigo et al., 1993; Farris et al., 1994, 1995; Larson, 1994), none has received more attention than the ILD test. The origins of the test stem from the homoplasy index proposed by Mickevich and Farris (1981). That index was designed to measure the degree to which homoplasy is increased by combined parsimony analysis over the levels already present in the individual data sets. For example, in the analysis of two data matrices, $\boldsymbol{X}$ and $\boldsymbol{Y}$, the index identifies a value (D_{xy}) that is derived by subtracting the combined length of the individual trees ($L_x + L_y$) from the length of the combined tree (L_{x+y}). As homoplasy is increased by combined data analysis, the value of D will increase. But that begs the question as to what is the statistical significance of a large D.

A statistical test was independently derived by Swofford and by Farris to determine the significance of D values. Farris et al. (1994, 1995) described it as an incongruence length difference test, and Swofford called it a "combinability" test in the earliest versions of PAUP* (the name was soon changed to the partition homogeneity test for reasons that will be discussed later). In both cases, the test compares an observed D against a null distribution generated by randomizing data into partitions of sizes equal to the original partitions. D is calculated for the observed partitions and all random partitions some number of times, represented by W. Then, the number of replicates for which D from the random partitions is less than the observed partition is calculated and designated S. A P-value for determining the probability of rejecting the null hypothesis of congruence (or homogeneity) is equal to $1-S/W$.

TABLE 1. Abbreviations and GenBank accession numbers for study taxa. Slow lorises are highlighted with bold font. "f" for female; "m" for male.

Binomial	Source of tissue	GenBank accession no.	
		Cytochrome b	IRBP
Lorisiformes			
Galago matschiei	FMNH[a] 148985; Burundi	AF271409	AF271414
Galago moholi	DUPC[b] 2006f	AF271410	AF271415
Galagoides demidoff	DUPC 3062f	AF271411	AF271416
Otolemur crassicaudatus	DUPC unknown	U53579[c]	Z11805[d]
Otolemur garnetti	DUPC 8030m	AF271412	AF271417
Loris tardigradus	DUPC 1966m	U53581[c]	AF271418
Nyticebus coucang	DUPC 1942f	U53580[c]	AF271419
Perodicticus potto	FMNH 148987; Burundi	AF271413	AF271420
Lemuriformes/Outgroups			
Cheirogaleus major	DUPC 639m	U53570[c]	AF271421
Daubentonia madagascariensis	DUPC 6262f	U53569[c]	AF271422
Lemur catta	DUPC 5738m	U53575[c]	AF081058[e]
Propithecus tattersalli	DUPC 6196m	U53573[c]	AF081053[e]
Tarsius bancanus	DUPC 52f (for IRBP)	AB011077[f]	AF271423
Saimiri sciureus	SIS 8445f; Tulsa Zoo	U53582[c]	AF271424
Homo sapiens	Unknown	J01415[g]	J05253[h]
Mus domesticus	Unknown	J01420[i]	Z11813[d]

[a] Field Museum of Natural History.
[b] Duke University Primate Center.
[c] Yoder et al., 1996.
[d] Stanhope et al., 1992.
[e] Yoder & Irwin, 1999.
[f] Andrews et al., 1998.
[g] Anderson et al., 1981.
[h] Fong et al., 1990.
[i] Bibb et al., 1981.

MATERIALS AND METHODS

Data Sets

Sources for DNA sequences are shown in Table 1, with primate taxonomy according to Groves (1993). Except for the *Mus domesticus* and *Homo sapiens* outgroup sequences (taken from GenBank), all sequences were derived from DNA extracted with a standard phenol/chloroform technique after digestion overnight in a sodium dodecyl sulfate–based extraction buffer. Tissue samples for the majority of strepsirrhine taxa were taken from animals that died of natural causes at the Duke University Primate Center (DUPC). Two animals, *Galago matschiei* and *Perodicticus potto*, were collected in the field by Julian C. Kerbis and are accessioned to the Field Museum of Natural History (FMNH 148985 and 148987, respectively). Amplification and sequencing conditions for the entire 1,140-bp cytochrome *b* gene were as described in Yoder et al. (1996), except that additional primers were designed to avoid coamplification of nuclear pseudogenes in the family Galagonidae (genera *Otolemur, Galago*, and *Galagoides*). The pseudogene-excluding primers are L14610 (CCC CCA TAA ATA GGA GAA GGC TT), which lies in the *NADH5* gene, and H16540 (CCA TCG TGA TGT CTT ATT TAA GGG GAA CGT), which lies in the mitochondrial control region. From exon 1 of the interphotoreceptor retinoid binding protein (IRBP) 939 bp was amplified and sequenced with the primers and conditions listed in Table 2. Polymerase chain reaction (PCR) products were cycle-sequenced by using a dye terminator sequencing kit (Applied Biosystems, Foster City, CA) and then analyzed by gel electrophoresis with an Applied Biosystems automated DNA sequencer (model 377). Sequences were edited and compiled with AutoAssembler 1.3.0 (Applied Biosystems) and are available in GenBank under the accession numbers listed in Table 1. Alignment of DNA sequences for both genes was obvious because of the lack of insertions and deletions and was performed by eye.

An 89-character morphological data set was analyzed separately and in combination with the molecular data. Characters 1–85 of this data set are equivalent to characters 1–86 (minus character 9) reported in the appendix to Yoder (1994). Character 9 was removed because it is invariant for the present taxon sample. In addition, four more characters were scored for the present study: 86, number of thoracic vertebrae (state 0 = less than or equal to 13, state 1 = more than or

TABLE 2. IRBP primers. "p" indicates 5′ (forward) primer, "m" for 3′ (reverse) primers; numbering is relative to sequences published in Stanhope et al. (1992).

Primer	Sequence											Annealing temp, °C
p141	CTG	GTC	ATC	TCC	TAT	GAG	CCC	AGC	A			65
p217	ATG	GCC	AAG	GTC	CTC	TTG	GAT	AAC	TAC	TGC	TT	55
p379	CCT	CGC	CTG	GTC	ATC	TCC	TAT	GAG	CCC	AGC	AC	55
p545	CCA	GGT	CCT	GGG	AGA	GAG	GTA	TG				65
p555	CTG	GGA	GAG	AGG	TAT	GGT	GCC	GAC	AA			65
p771	CTT	GGT	GGA	GGC	AGC	CAG	ACG	TGG	GA			65
m1531	CGC	AGG	TCC	ATG	ATG	AGG	TGC	TCC	GTG	TCC	TG	55
m1208	TCA	GCA	AAG	CTG	TCG	AAG	CGC	AGG	TA			65
m977	GCG	TTG	AGC	TTG	GTG	ACC	AGA	TCC	T			65
m697	ACG	GTG	AGG	AAG	AAG	TTG	GAT	TGG				65
m417	TGC	AGG	TAG	GAG	ATG	ATG	TAG	GGA	ATG	C		65

equal to 15; Yoder, 1992); 87, absence (state 0) or presence (state 1) of retia mirabilia of the proximal limb vessels (Wislocki and Straus, 1932; Ikeda et al., 1988, 1992); 88, absence (state 0) or presence (state 1) of a longitudinal septum in the auditory bulla (Rasmussen and Nekaris, 1998); and 89, no anteromedial expansion of the ethmoturbinals (state 0) or anterior and medial expansion of the ethmoturbinals (state 1; Eaglen, 1980). Character state distributions for morphological characters 86–89 are illustrated in Table 3; all others are available in Yoder (1994).

Methods of Analysis

PAUP* 4.0b2a (PPC) was used for all phylogenetic analyses as well as for bootstrap and partition homogeneity tests (Swofford, 1998). Parsimony analyses of the individual and combined molecular data sets were conducted with 100 replicates of the random addition heuristic search option with TBR branch swapping. The branch and bound algorithm was used for the morphological and for the combined molecular and morphological analyses because of the smaller taxon sample. To test for data set incongruence, we used the partition homogeneity test executable in PAUP* (this test is equivalent to the incongruence length difference test of Farris [Farris et al., 1994, 1995]; for convenience, it will be referred to as the ILD in this paper). We conducted 1,000 replicates of the ILD test (using either the random addition heuristic search option or the branch and bound option, depending on taxon sample, as described above). Additional replicates were run (up to 100,000) if accurate *P*-values could not be determined from 1,000 replicates. Parsimony-uninformative characters were removed before each test. These analyses were conducted for both the maximum taxon sample possible (which differs for the molecular and morphological data sets) and for a smaller taxon sample selected to focus on potential phylogenetic conflict within the Loridae. This latter sample includes only nine taxa: *Otolemur crassicaudatus, Galago moholi, Galagoides demidoff, Perodicticus potto, Nycticebus coucang, Loris tardigradus, Daubentonia madagascariensis, Propithecus tattersalli, and Lemur catta*.

Molecular characters were analyzed with maximum parsimony under both equal-weighting and various transversion-weighting conditions. Transversion weights were determined based on "classic" estimates of transition/transversion (i/v) ratio (terminology introduced by Wakeley [1996]), as well as by maximum likelihood estimates of i/v ratio. For the latter, estimates

TABLE 3. Morphological characters specific to this study. Slow lorises are highlighted with bold font. Characters 1–85 (not shown) are from Yoder (1994).

	Characters[a]			
Binomial	86	87	88	89
Galago moholi	0	0	1	1
Galagoides demidoff	0	0	1	1
Otolemur crassicaudatus	0	0	1	1
Loris tardigradus	1	1	1	1
Nyticebus coucang	1	1	1	1
Perodicticus potto	1	1	1	1
Cheirogaleus major	0	0	0	0
Daubentonia madagascariensis	0	0	0	0
Lemur catta	0	0	0	0
Propithecus tattersall	0	0	0	0
Tarsius bancanus	0	0	0	0
Saimiri sciureus	0	0	0	0

[a]Character descriptions are given in Materials and Methods section.

were determined separately for the complete taxon sample and for the reduced taxon sample because both empirical and computer simulation studies have shown that i/v estimates are sensitive to taxon sample (Yang and Yoder, 1999). Several weighting schemes were explored in which morphological characters were up-weighted to account for imbalance in number of informative characters or tree length between morphological and molecular data sets. Bootstrap analysis was used as a measure of node support in all parsimony analyses. For analyses conducted for the maximum taxon sample, 100 bootstrap replicates were run with the random addition option (10 addition replicates per bootstrap replicate). For the smaller taxon sample, 100 bootstraps were run with branch and bound. For all parsimony analyses, random seed numbers were set to 1 to assure repeatability.

Data decisiveness (sensu Goloboff, 1991) was determined for the reduced taxon sample. The method uses the following equation to measure decisiveness,

$$\mathrm{DD} = \frac{\bar{S} + S}{\bar{S} + M}$$

where $\bar{S}$ is the mean length of all possible trees, S is the length of the most parsimonious tree(s), and M is the sum of the minimum possible number of character state changes for a given data set (i.e., the length of the perfectly nonhomoplasious tree). The exhaustive search option in PAUP* was used to calculate $\bar{S}$ and S; the character diagnostics option of the Describe Trees submenu was used to calculate M.

The molecular data sets were also analyzed by maximum likelihood in PAUP* with parameter settings selected by MODELTEST (Posada and Crandall, 1998). The Kishino–Hasegawa test (Kishino and Hasegawa, 1989) was used to evaluate the extent to which the maximum likelihood tree is significantly better than competing trees under a given model. Partitioned likelihood analysis was also performed by using MODELTEST to determine likelihood parameters for each codon position subset individually. Because PAUP* cannot search tree space under a partitioned likelihood model, a large set of candidate trees was evaluated for each of the three codon subsets with likelihood values summed across codon subsets to determine the most likely tree. As many as 2,000 candidate trees, including the trees chosen by the parsimony and likelihood optimality criteria, were collected under constrained and reverse-constrained conditions of slow loris monophyly. Estimates of node support under maximum likelihood were calculated in PAUP* by quartet puzzling (Strimmer and von Haesler, 1996; Strimmer et al., 1997; Cao et al., 1998).

RESULTS

Data Set Characteristics

The data sets differ considerably in the number of parsimony-informative characters contained by each. Cytochrome *b* contains the majority, followed by IRBP, and then morphology. For the complete combined taxon sample, cytochrome *b* contains 480 parsimony-informative characters, IRBP has 181, and the morphological data set only 71. For the smaller taxon sample, these numbers decline to 354 for cytochrome *b*, 81 for IRBP, and 57 for the morphological data set. Therefore, in the complete and reduced molecular and morphological combined analyses, informative molecular characters outnumber morphological characters by nearly 10:1 and 8:1, respectively. The data sets also differ markedly in measures of data decisiveness (Goloboff, 1991). The morphological and IRBP data sets were both highly "decisive," with DDs of 0.83 and 0.81, respectively. Cytochrome *b*, on the other hand, had a low DD (0.22). The statistics used to determine these values are as follows: morphology—$\bar{S} = 179$, $S = 115$, $M = 102$; IRBP—$\bar{S} = 306$, $S = 213$, $M = 191$; and cytochrome *b*—$\bar{S} = 1281$, $S = 1159$, $M = 733$.

For cytochrome *b*, a general time reversible model with an asymmetric nucleotide rate matrix and an i/v of 4.84 was preferred under both the hierarchical likelihood ratio tests (hLRTs) and the Akaike Information Criterion (AIC). The proportion of invariable sites was estimated at 0.3947 and the gamma distribution shape parameter at 0.9086. For the smaller taxon sample, the estimated rate for mutation classes differed considerably (e.g., $C \leftrightarrow T = 34.6262$ for the complete sample and 61.7548 for the reduced sample) but the difference in the rate ratio was negligible (4.84 v. 4.75), as were differences in other parameter estimates. For IRBP, the hLRT and

AIC differed in their selection of likelihood models. The hLRT selected the HKY + Γ with an estimated i/v ratio of 2.65 and gamma shape parameter of 0.4114, whereas the AIC selected a general time reversible model with asymmetric nucleotide rate matrix and estimated i/v of 2.79 and gamma shape parameter of 0.4054. Both methods estimated the proportion of invariable sites to be 0.0. For a combined cytochrome *b* plus IRBP data set, the hLRT and AIC both selected a general time reversible model with an asymmetric nucleotide rate matrix. The proportion of invariable sites was estimated at 0.3741 and the gamma distribution shape parameter at 0.8105.

Molecular Data: Analyzed Alone and in Combination

Parsimony analysis of the two molecular data sets, whether analyzed separately or in combination, fails to support the traditional hypothesis of slow loris monophyly (Fig. 1). For all three analyses in which characters were equally weighted, the results indicate that the family Loridae is paraphyletic. Consistently, the Asian slow lorises (*Nycticebus coucang* and *Loris tardigradus*) are shown to form a sister clade to the galagos, to the exclusion of the African slow loris, *Perodicticus potto*. Our results are therefore consistent with those reported in previous molecular phylogenetic analyses (Sarich and Cronin, 1976; Porter et al., 1997; Goodman et al., 1998). *P. potto*'s basal position in the lorisiform tree, however, appears to give no support for Grove's (1971) biogeographic hypothesis of African lorisiform monophyly to the exclusion of the Asian slow lorises. If Grove's hypothesis were true, then *P. potto*, rather than *N. coucang* and *L. tardigradus*, would be expected to join the galago clade.

Numerous studies have shown that transitions occur more frequently than transversions during molecular evolution (Brown et al., 1982; Jukes, 1987; Tamura and Nei, 1993; Wakeley, 1996; Huelsenbeck and Neilsen, 1999), a finding used as a rationale for various modes of transversion weighting in parsimony analysis (e.g., Chippindale and Wiens, 1994; Yoder et al., 1996; Voelker and Edwards, 1998). We used maximum likelihood estimates of the i/v ratio, rounded to the nearest whole number, to determine the transversion weights for parsimony analysis. When transversions are weighted fivefold more heavily than transitions (i/v 5 weighting) in the cytochrome *b* data set, the relative positions of *P. potto* and the Asian slow

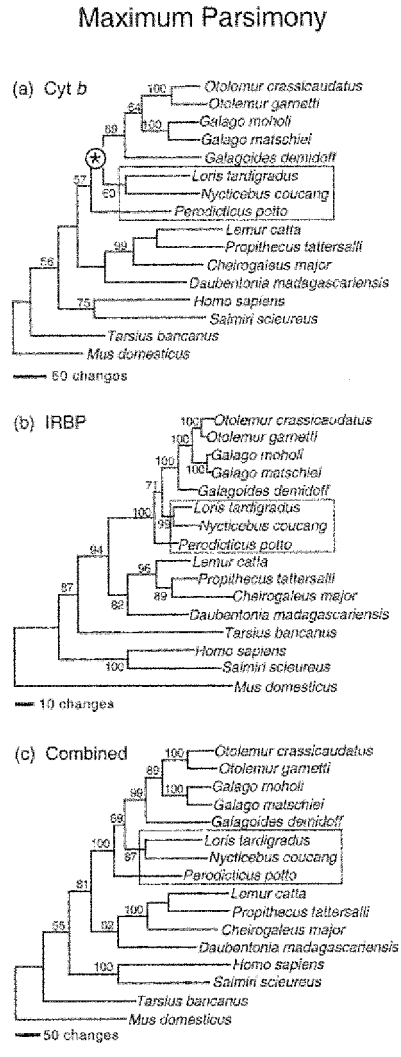

FIGURE 1. Maximum parsimony trees for separate and combined analyses of cytochrome *b* and IRBP in which all characters are equally weighted. Trees are rooted with *Mus domesticus* as the outgroup. Numbers on branches represent bootstrap values. Branches are drawn to be proportional to the number of inferred character state changes. Boxes highlight resolution of slow loris taxa. (a) Individual analysis of cytochrome *b*; one of two trees of length 2,122, CI = 0.458, retention index [RI] = 0.364. (b) Individual analysis of IRBP (exon 1); one tree of length 612, CI = 0.752, RI = 0.705. (c) Combined analysis of cytochrome *b* and IRBP; one tree of length 2,742, CI = 0.522, RI = 0.436. The asterisk (*) indicates the node discussed throughout this paper as supporting slow loris paraphyly.

lorises reverse (results not shown). In this case, *P. potto* is inferred with moderate bootstrap support (67%) to be the sister taxon to the galagos. Weighting transversions threefold more heavily in the IRBP data set (i/v 3 weighting) does not alter the unweighted tree topology; the resulting tree is identical to that illustrated in Figure 1b but with less bootstrap support (58%) for the node supporting a sister group relationship between the Asian slow lorises and the galagos. The combined analysis of the transversion-weighted data lends further support to the weighted cytochrome *b* analysis, however: In the combined transversion-weighted analysis, *P. potto* is again placed as sister to the galagos, although with only moderate (64%) bootstrap support.

In our experience, weak or conflicting phylogenetic results such as those described above are often related to short internal branches, associated either with rapid evolutionary radiation or with rate acceleration after lineage divergence. In either case, maximum likelihood analysis is often recommended as a means for improving phylogenetic accuracy (Felsenstein, 1978). Using the parameters selected by MODELTEST, we conducted maximum likelihood analyses for the two molecular data sets individually and in combination (Fig. 2). The results with regard to slow loris phylogeny are identical to those of the transversion-weighted parsimony analysis. Both the cytochrome *b* and combined analyses place *P. potto* sister to the galagos, whereas the IRBP analysis places it basally. Inspection of relative branch lengths confirms the suspicion of short internal branches resolving the relative placement of the slow lorises, particularly for the IRBP data set (Fig. 2b). Interestingly, however, when tree topologies were evaluated under partitioned likelihood models (i.e., parameters were estimated individually for each codon subset), the IRBP data favored a tree showing slow loris monophyly as the most likely tree for both the complete (Fig. 3a) and the smaller taxon samples (not shown). In contrast, the cytochrome *b* data continued to indicate slow loris paraphyly for both taxon samples (illustrated for the complete taxon sample in Fig. 3b). However, the cytochrome *b* data under partitioned likelihood placed the Malagasy lemuriforms in a clade with the two anthropoid taxa sampled (*Homo* and *Saimiri*), thereby casting doubt

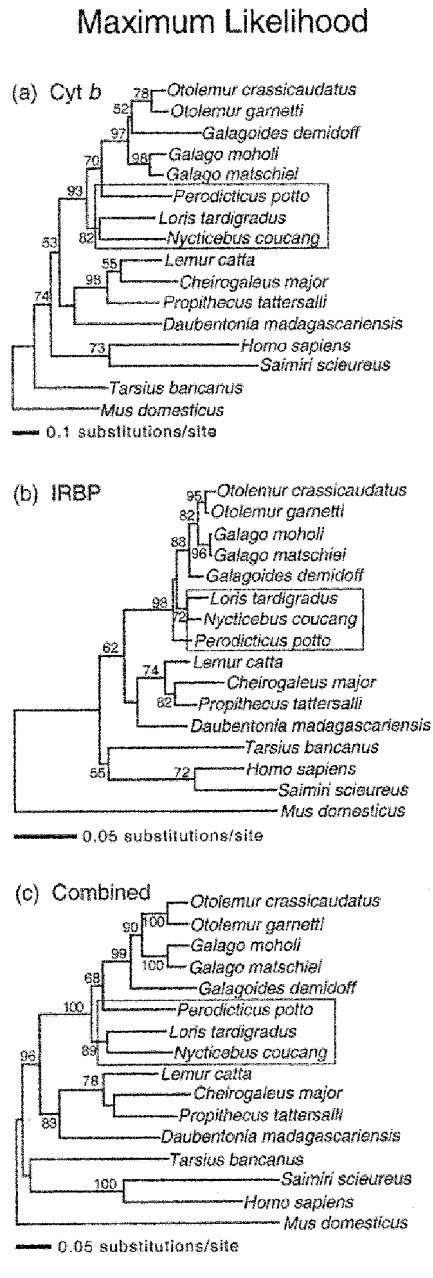

FIGURE 2. Maximum likelihood trees for separate and combined analyses of cytochrome *b* and IRBP. Likelihood parameters were set as discussed in Results. Branch lengths are drawn to be proportional to the expected number of changes per site for that branch. Numbers on branches represent quartet puzzling values. Boxes highlight resolution of slow loris taxa. (a) Individual analysis of cytochrome *b*; −Ln likelihood = 9,429.6262, estimated i/v ratio = 4.84, estimated gamma shape parameter = 0.9086. (b) Individual analysis of IRBP; −Ln likelihood = 4,242.9952, estimated i/v ratio = 2.79, estimated gamma shape parameter = 0.4054. (c) Combined analysis of cytochrome *b* and IRBP; −Ln likelihood = 14,132.8105, estimated i/v ratio = 2.92, estimated gamma shape parameter = 0.8105.

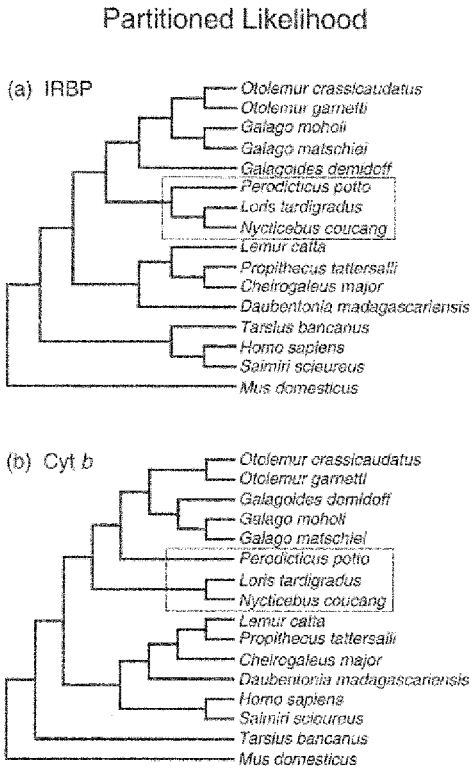

FIGURE 3. Maximum likelihood trees from analysis in which likelihood parameters were selected for individual codon sets (i.e., first positions only; second positions only; third positions only). Likelihood parameters for cytochrome *b* are: first position—i/v ratio = 2.18, proportion of invariable sites = 0.0, gamma distribution shape parameter = 0.2265; second position—i/v ratio = 4.10, proportion of invariable sites = 0.5897, gamma distribution shape parameter = 0.6453; third position—i/v ratio = 37.38, proportion of invariable sites = 0.0090, gamma distribution shape parameter = 0.6622. Likelihood parameters for IRBP are; first position—i/v ratio = 2.26, proportion of invariable sites = 0.0, gamma distribution shape parameter = 0.3539; second position—i/v ratio = 2.31, proportion of invariable sites = 0.0, gamma distribution shape parameter = 0.1697; third position—i/v ratio = 2.42, proportion of invariable sites = 0.0, gamma distribution shape parameter = 2.1396. (a) Tree selected by IRBP partitioned data as the most likely of 1,767 candidate trees; summed −Ln likelihood across three codon subsets, 4,002.3731. Boxes highlight resolution of slow loris taxa. (b) Tree selected by cytochrome *b* partitioned data as the most likely of 1,900 candidate trees; summed −Ln likelihood across three codon subsets, 8,797.3609.

on the accuracy of these results. Moreover, the Kishino–Hasegawa test indicates that the most likely tree for each data set is not significantly more likely than trees that are otherwise identical except for differing resolution of the slow lorises.

Morphological Data: Analyzed Alone and in Combination with Molecular Data

As described in the introduction, the list of physical characteristics that appear to define a slow loris clade is extensive. Not surprisingly, therefore, parsimony analysis of the morphological data set yields a hypothesis of monophyly with 100% bootstrap support (Fig. 4a). Inspection of reconstructed character-state changes on the tree reveals 16 morphological apomorphies supporting the slow loris clade, 7 of which are unreversed (consistency index [CI] = 1.00). All other strepsirrhine nodes resolved by the morphological analysis are compatible with those defined by the molecular analyses. Like the molecular data, the morphological data recover a Malagasy lemuriform clade, a lorisiform clade, and a galago clade. Moreover, all three clades are supported by moderate (65%) to high (89%) bootstrap values. Apparently, therefore, the only potential conflict between the molecular and morphological data sets is confined to the differing resolutions of the slow loris group.

When the molecular data are added to the morphological data in an equally weighted combined analysis (Fig. 4b), bootstrap support for a slow loris clade declines markedly, although at 74%, the support can still be termed persuasive (Hillis and Bull, 1993). When evaluating this result, however, remember that the phylogenetically informative characters contained within the molecular data outnumber those within the morphological data set by at least eight to one (depending on taxon sample), thereby offering potential justification for a differential weighting scheme in which morphological characters receive eight times the weight of the molecular characters (Miyamoto, 1985). Even so, when morphological characters are given a weight only twice that of molecular characters, bootstrap support for the slow loris clade climbs to 96%. The results of the combined morphological and molecular analyses therefore provide robust support for the hypothesis of slow loris monophyly, contrary to the molecular-only results presented here and elsewhere in the literature.

Incongruence, Bootstrap Support, and Phylogenetic Accuracy

Results such as those described above, wherein different data sets yield different

phylogenetic resolution of the same taxa, invite speculation that data partition conflict or heterogeneity may be in effect. Although problems such as sampling error (Bull et al., 1993; Huelsenbeck et al., 1996) or inadequacies of the reconstruction model (Cunningham, 1997a) might potentially explain the disagreement, and thus not preclude combined data analysis, more severe problems of a biological nature (i.e., the data sets represent more than one history) are also suspect. In the latter case, data set combination is not advisable. We performed a comprehensive suite of combined analyses, investigating data set incongruence as measured by the ILD test, to investigate the hypothesis that data set heterogeneity may be causing differential resolution of the slow loris taxa. We also measured clade support by bootstrap analysis for each of the combined data sets. All data combinations were analyzed under both equally weighted and transversion-weighted conditions. In all cases, two different taxon samples were investigated: a complete taxon sample (as illustrated in Figs. 1–3 for the molecular data and in Fig. 4 for the morphological and combined molecular and morphological data) and a smaller taxon sample (in which all nonstrepsirrhine outgroups and the genus *Cheirogaleus* were removed, as were the species *G. matschiei* and *O. garnettii* when present). The motivation for conducting the analyses and tests with a smaller taxon sample was both to focus on incongruence related to the slow lorises and to assure that taxon sampling was identical and thus comparable in all combined analyses.

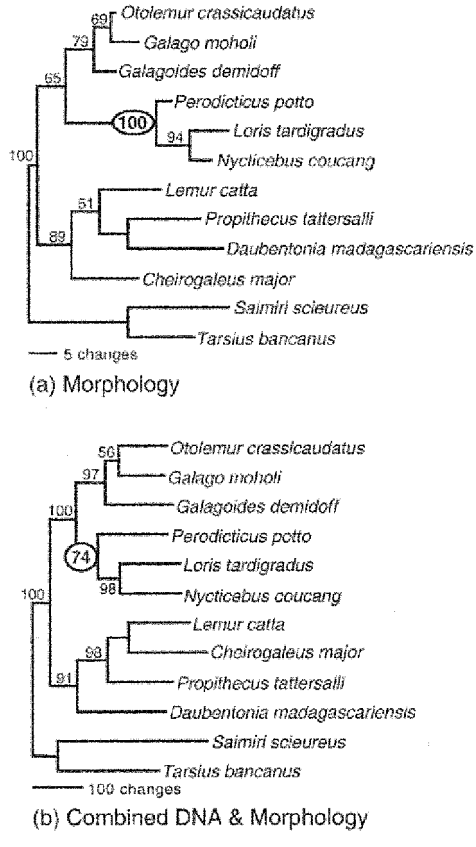

FIGURE 4. Maximum parsimony trees for separate analysis of morphological data and equally weighted analysis of combined morphological, cytochrome *b*, and IRBP data. Numbers on branches represent bootstrap values. Circled bootstrap value highlights strength of resolution of slow loris clade. (a) Individual analysis of morphological data: one of two trees of length 177, CI = 0.661, RI = 0.661. (b) Combined analysis of morphological and genetic data: one tree of length 2,288, CI = 0.580, RI = 0.425.

Results for the smaller taxon sample are shown in Table 4. The various data combinations and weighting schemes are indicated, along with associated *P*-values from the ILD tests and resolution of the slow lorises with bootstrap support (i.e., whether they were found to be monophyletic or paraphyletic in that analysis). Bootstrap support for a paraphyletic resolution is identified for the node that supports a sister group relationship between the galagos and some, but not all, of the slow lorises (e.g., the node indicated with an asterisk in Fig. 1b). Reducing the taxon sample tends to increase incongruence. Whereas only two of the complete taxon analyses yielded *P*-values <0.05, (both of which are for the combined IRBP and morphology data [not shown]), the majority of the smaller taxon analyses yielded *P*-values considerably less than 0.05 (Tables 4 and 5). We interpret this result as an indication that the reduced taxon sample, by design, focuses the ILD test on incongruence specific to the slow lorises. This follows the reasoning of Thornton and DeSalle (2000), who showed that incongruence can be differentially represented by isolated clades within a larger phylogeny. Alternatively, the apparent increase in heterogeneity might be a simple function of a smaller sample size, regardless of the taxonomic composition of that sample. To test the latter hypothesis, we conducted ILD tests for the same data set combinations and weighting schemes, but

TABLE 4. Comparison of ILD and bootstrap results for reduced (smaller) taxon sample. 3rd tv refers to third-position transversions only (after Yoder et al., 1996); i/v 5 and i/v 3 refer to transversion weighting based on maximum likelihood estimates of transition/transversion ratio for cytochrome *b* and IRBP, respectively; dashes indicate which partition was omitted; monophyly and paraphyly refer to resolution (or not) of slow loris clade; bootstrap values are given accordingly. Bold text highlights case where strongest indication of partition heterogeneity is associated with greatest bootstrap support for slow loris monophyly.

	Partitions				
	Morphology	Cytochrome *b*	IRBP	ILD *P*-value	Lorid bootstraps
1)	Equal	Equal	Equal	0.004	Monophyly, 85%
2)	Equal	Equal	—	0.015	Monophyly, 90%
3)	Equal	3rd tv	—	0.004	Monophyly, 97%
4)	Equal	—	Equal	**0.00005**	**Monophyly, 100%**
5)	Equal	—	i/v 3	0.001	Monophyly, 96%
6)	Equal	i/v 5	i/v 3	0.664	Paraphyly, 49%
7)	Equal	i/v 5	—	0.333	Paraphyly, 52%
8)	—	Equal	Equal	0.329	Paraphyly, 63%
9)	—	i/v 5	i/v 3	0.978	Paraphyly, 43%

with different taxa in the smaller taxon sample. For example, in the combined equally weighted analysis of all three data sets (first line of Table 4), we removed the three slow loris taxa, thereby yielding an identical number (but different combination) of taxa in a reduced sample. The results uphold the conclusion that the original smaller taxon sample focused on incongruence in the Loridae because the *P*-value increased from 0.004 to 0.619 in the revised smaller taxon. Thus, there is considerably less incongruence when the smaller taxon sample does not include the slow lorises.

Although reducing the taxon sample tends to focus on heterogeneity relating to slow loris phylogeny, it has no effect on phylogenetic resolution. In other words, the analyses with fewer taxa also resolve the same relevant clades recovered in the complete taxon analyses. Both taxon samples resolve a lorisiform clade (node A in Fig. 5), an African bushbaby clade (node B in Fig 5), and a lemuriform clade (node C in Fig. 5). Moreover, these same clades are resolved regardless of the weighting scheme used. In fact, Figure 5 is the strict consensus phylogeny of the nine combined analyses listed in Table 4. The only area of disagreement relates to the slow loris group (highlighted with a box in Fig. 5). We argue, therefore, that *P*-values can be interpreted as directly reflective of phylogenetic

TABLE 5. Effects of differential weighting on ILD, bootstrap, and tree statistics. i/v refers to transversion weighting. M = monophyly and P = paraphyly with respect to resolution (or not) of slow loris clade. D_{xy} is homoplasy index of Mickevitch and Farris (1981). TL = tree length; CI = consistency index. All values were calculated after uninformative characters were excluded. Results shown are from the smaller taxon sample.

Morphology weight	TL (L_x)	CI_x	Molecular weight	TL (L_y)	CI_y	Combined TL (L_{x+y})	CI_{x+y}	D_{xy}	Combined bootstrap	ILD *P*-value
Cytochrome *b*										
1×	91	0.725	Equal	969	0.560	1069	0.570	9	M = 90%	0.015
6×	546	0.725	Equal	969	0.560	1541	0.609	26	M = 100%	0.0007
10×	910	0.725	Equal	969	0.560	1907	0.631	28	M = 100%	0.008
1×	91	0.725	i/v 5	2511	0.559	2619	0.561	17	P = 52%	0.333
6×	546	0.725	i/v 5	2511	0.559	3102	0.582	45	M = 98%	0.0005
28×	2548	0.725	i/v 5	2511	0.559	5147	0.632	88	M = 100%	0.003
1×	91	0.725	i/v 10	4406	0.557	4514	0.558	17	P = 58%	0.818
6×	546	0.725	i/v 10	4406	0.557	5027	0.567	75	M = 66%	0.004
48×	4368	0.725	i/v 10	4406	0.557	8942	0.629	168	M = 100%	0.002
IRBP										
1×	91	0.725	Equal	116	0.810	212	0.755	5	M = 100%	0.00005
2×	182	0.725	Equal	116	0.810	330	0.770	32	M = 100%	0.0003
1×	91	0.725	i/v 2	151	0.821	247	0.769	5	M = 98%	0.0004
2×	182	0.725	i/v 2	151	0.821	345	0.751	12	M = 100%	0.0003
1×	91	0.725	i/v 3	186	0.830	282	0.780	5	M = 96%	0.001
2×	182	0.725	i/v 3	186	0.830	376	0.761	8	M = 100%	0.001

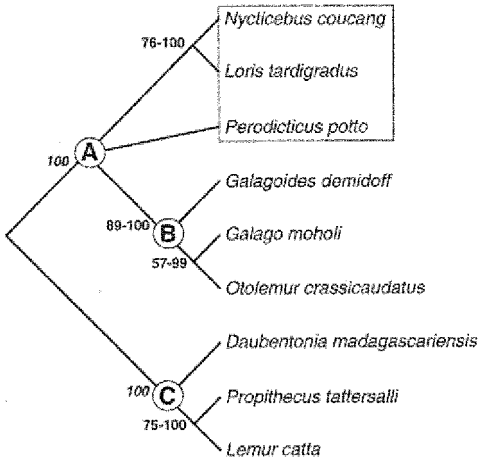

FIGURE 5. Strict consensus of maximum parsimony trees derived from combined analyses described in Table 4. Numbers indicate range of bootstrap values from all analyses. Midpoint rooting was used to determine root of tree. Note that 100% bootstrap support for the separation of the lorisiform and lemuriform clades occurs only once in the unrooted network but is illustrated twice (shown in italics) on the rooted tree for graphical emphasis. Box highlights resolution of slow loris taxa. Node A = Afroasian lorisiforms; Node B = African galagos; Node C = Malagasy lemuriforms.

conflict relating to the resolution of the slow lorises.

P-values vary widely in Table 4, from 0.978 to 0.00005, suggesting that some data/weighting combinations are not in conflict but others are. The impact of incongruence on phylogenetic accuracy is completely counterintuitive, however. If we assume that the slow loris clade is real, and thus monophyly is the accurate result, then phylogenetic accuracy and lack of conflict have a nearly inverse relationship. In other words, in cases where incongruence is low ($P > 0.01$), a presumably incorrect result of slow loris paraphyly typically is found. On the other hand, in all cases in which incongruence is high ($P \leq 0.01$), a presumably correct result of monophyly is found, always with strong statistical support (bootstrap $\geq$ 85%). In fact, the cases of strongest incongruence ($P = 0.00005$) yield accurate results with the most support (bootstrap = 100%).

DISCUSSION

What Is the ILD Good For?

The test was originally conceived as a method for determining whether data sets should be combined in a single parsimony analysis, as is obvious from its original description as a "combinability" test in early versions of PAUP*. However, the name was later changed to "partition homogeneity" because of the realization that the test was inappropriate for deciding whether data sets should be combined (David Swofford, pers. comm.). The name change recognizes the possibility that combining data sets for which partition homogeneity is rejected might still yield more accurate phylogenetic estimates, just as a partitions that are deemed "homogeneous" may still contain substantial amounts of among-subset incongruence (release notes for PAUP* version 4.0d50, Oct. 1996). Nonetheless, investigators have continued to use the test to determine data set heterogeneity and thus, presumably, noncombinability. Even so, the test's perceived sensitivity has undergone a steady decline. When the ILD was originally developed, Farris recommended a *P*-value of 0.05 as the threshold for determining noncombinability. Since then, numerous investigators have applied the test to empirical data and have found that *P*-values < 0.05, and even as low as 0.001, should not preclude data set combination (Sullivan, 1996; Cunningham, 1997a,b; DeSalle and Brower, 1997; Sidall, 1997; Davis et al., 1998; Flynn and Nedbal, 1998; Messenger and McGuire, 1998).

So how do our results compare with those of previous studies? For one, they appear to support the idea that character weighting can markedly reduce incongruence (Cunningham, 1997a). For example, transversion weighting in the combined morphology and DNA data set for the reduced taxon sample increased *P*-values from 0.004 (Table 4, line 1) to 0.664 (Table 4, line 6), indicating a substantial decrease in incongruence. In this case, however, reduced incongruence did not increase accuracy. On the contrary, accuracy was diminished. Whereas the equally weighted analysis recovered the slow loris clade, the weighted analysis did not. Indeed, our study appears to indicate an inverse relationship between congruence and accuracy. In all cases where incongruence was severe ($P \leq$ 0.004), accuracy was actually enhanced (i.e., the Loridae were shown to be monophyletic) and bootstrap support for that clade was high ($\geq$85%). The most dramatic example was the equally weighted morphology plus IRBP analysis (Table 4, line 4). In that case,

support for lorid monophyly is ideal (bootstrap = 100%) though incongruence is extreme ($P = 0.00005$) Thus, our study continues the trend that has found diminishing sensitivity of the ILD test, although the complete reversal of congruence and accuracy is unexpected and difficult to explain.

Effects of Weighting on Incongruence

As mentioned above, transversion weighting appears to decrease incongruence, but at an apparent cost of diminished accuracy, particularly in the cytochrome *b* data set. In all but the case in which third-position-only transversions were combined with the morphological data (Table 4, line 3), transversion weighting in the cytochrome *b* data set yielded weak support for the wrong phylogeny. This suggests that transversion weighting might be overwhelming the morphological signal if the morphological data are not upweighted accordingly (Cliff Cunningham, pers. comm.). To investigate the effects of character weighting on data set incongruence and slow loris resolution, we compared the equally weighted combined analyses with those in which transversions were weighted according to either maximum likelihood or classical method (Brown et al., 1982; Jukes, 1987) estimates of i/v ratio; morphological characters were variously upweighted to account for imbalance in informative characters (6× for cytochrome *b*; 2× for IRBP) or for imbalance in partition tree lengths. For the latter, tree length was estimated for the morphological data alone and also for the transversion-weighted molecular data alone. Thus, because the i/v 5 weighted cytochrome *b* tree is ~28 times longer than the unweighted morphological tree, the morphological data were upweighted by 28×.

Inspection of Table 5 shows that this weighting achieved one of the desired effects, in that individual tree lengths were approximately balanced (2,548:2,511 with upweighting v. 91:2,511 without upweighting). However, the results of the upweighting experiment merely reinforce the previous observation of inverse relationship between congruence and accuracy. Whenever the accurate phylogeny receives strong statistical support, *P*-values tend to be <0.01, usually markedly so, indicating significant incongruence. Conversely, in the two cases in which *P*-values are >0.05, slow loris paraphyly rather than monophyly is recovered. In both of these cases, tree lengths of the individual morphological and molecular data sets are markedly divergent, the molecular tree length far exceeding that of the morphological tree. This leads us to believe that congruence and lack of accuracy are simply a matter of the morphological data being overwhelmed by the transversion-weighted cytochrome *b* data. Such behavior is not observed for the IRBP data because the applied transversion weights are less extreme and because the number of parsimony-informative characters is more balanced between the morphological and IRBP data sets.

We readily admit that the relative weighting schemes discussed above are perhaps extreme. Individual data partitions will more times than not contain differing numbers of informative characters, yield trees of different lengths, or both. The rationale for weighting as an equalizing strategy is tenuous. Nonetheless, this excercise in compensatory weighting has forcefully demonstrated the impact of the morphological data in these combined analyses, which tends to be perceived by the ILD as incongruence. Given that the morphological data are probably resolving the lorid phylogeny correctly (as will be discussed more fully, later in this paper), we perceive this as a flaw in the test.

Relationship of Homoplasy to Incongruence and Accuracy

The ILD was designed to measure the effect of data combination on the levels of homoplasy. As a determinant of combinability, the test assumes that as homoplasy is increased, accuracy will decrease. We already know, however, that this assumption is erroneous. Previous studies have shown that homoplasy as measured by CI has virtually no relationship to phylogenetic accuracy (Sanderson and Donoghue, 1989). Our investigation into the relationship among CI, incongruence, and accuracy (Table 5) also indicates that homoplasy has little relationship to accuracy for this study. For example, the IRBP data analyzed alone have the greatest CI (0.810–0.830, depending on transversion weights) but never recover lorid monophyly in parsimony analyses. This phylogenetic result is also true for the cytochrome *b* data analyzed alone, which have the lowest CI (0.557–0.560). Likewise, combined analysis

of cytochrome *b* and morphology tends to increase homoplasy relative to morphology alone (i.e., CI decreases markedly), but homoplasy decreases in combined morphology and IRBP analyses (i.e., CI increases relative to morphology alone), even though both combinations tend to recover the accurate phylogeny.

Surprisingly, CI also appears to have little influence on significance levels of incongruence. If we examine the CI of the combined tree relative to the CI of the morphological tree, changes of anywhere from +6% to −22% are perceived to be incongruent ($P < 0.01$), whereas changes of −21% to −23% are tolerated as congruent. In other words, a large increase in homoplasy (indicated by a substantial decrease in CI) is better tolerated by the ILD than is a small decrease in homoplasy. Likewise, the magnitude of *D*, in and of itself, has little relationship to the significance of the incongruence. The only relationship that appears to have any predictable effect on incongruence is that the closer the CI of the combined analysis is to the CI of the molecular data, the less significant is the incongruence (as seen in any of the combined transversion-weighted cytochrome *b* analyses in which morphological characters were not upweighted). But, as already reported, this occurs only because the morphological data are being subsumed by the molecular data because of the vast imbalance in their relative tree lengths.

Character Support Versus Data Decisiveness

The discussion above solves only one-half of the puzzle. The correlation of paraphyly with lack of incongruence in the combined morphology and cytochrome *b* analysis is the result of excessive weighting of the molecular data when transversion weights are applied. Because the tree length of the molecular data so far exceeds that of the morphological data, the ILD does not detect a significant change in CI. And, because the cytochrome *b* data in isolation do find paraphyly, the relatively weak signal in the unweighted morphological data does not alter the molecular resolution.

But why do accuracy (i.e., lorid monophyly) and significant incongruence show a correlation? And why does this appear to be especially acute in the combined morphology and IRBP analyses? If the ILD is to be useful as a test of data combinability, we would hope it is testing an essential feature of phylogenetic conflict, and given that the test is parsimony-based, we would hope the perceived conflict would be related to conflicting character support for the opposing monophyletic and paraphyletic resolutions of the slow loris group. Oddly, this seems not to be the case for the morphology and IRBP combined analyses, in which the measures of incongruence are extreme. Despite the remarkable branch support for the lorid clade in the morphological tree (16 characters, 7 with CI = 1.0), there is very little character support for the paraphyletic resolution in the IRBP tree (four characters, two with CI = 1.0). When the two data partitions are combined, the same 16 morphological characters and two different IRBP characters support the slow loris clade. Remarkably, however, six of the seven morphological characters that had CI < 1.0 in the morphology-only analysis, become homoplasy-free in the combined analysis. Thus, in the combined analysis, 13 of the 16 morphological apomorphies are unreversed. Combined analysis of the IRBP and morphological data sets therefore seems to increase character support rather than character conflict for the problematic clade. On the other hand, cytochrome *b* branch support for the paraphyletic resolution, is apparently far more conclusive, with 50 characters (9 with CI = 1.0) supporting the branch that unites Asian slow lorises with galagos, to the exclusion of the African slow loris. Thus, the character conflict between the morphological and cytochrome *b* data could be potentially far more severe than that between the morphological and IRBP data. But this is not reflected in the ILD tests.

A possible explanation for the perceived incongruence between IRBP and morphology, and the seemingly milder conflict between morphology and cytochrome *b*, may be found in the respective "decisiveness" values (sensu Goloboff, 1991) among the three data partitions. Davis et al. (1998) warned that highly decisive data are more likely to be assessed as incongruent (and thus noncombinable) than are less decisive data sets. This prediction appears to be upheld by our results: The most extreme conflict occurs when morphology and IRBP, both of which have very high DD, are combined. When either is combined with the far less decisive cytochrome *b* data, ILD values are less

significant. Even so, DD does not fully explain the anomolous results, given that ILD values for cytochrome *b* (DD = 0.22) and morphology (DD = 0.83) indicate considerably more conflict than do combined cytochrome *b* and IRBP (DD = 0.81) analyses.

The Reality of a Slow Loris Clade

In this study, we have partitioned data such that each data set can be considered independent of the other two (i.e., mitochondrial, nuclear, and morphological partitions), have performed separate analyses of each, and have performed combined analyses under a variety of character-weighting schemes. In so doing, we have focused on an area of conflict specific to the resolution of the slow lorises. In effect, then, we have followed a methodology recently proposed by Wiens (1998) for analyzing data sets with different phylogenetic histories localized to a few taxa within the total taxonomic sample. By this methodology, we should consider the resolution of a slow loris clade to be tentative.

Clearly, however, all of the conclusions with regard to the reliability of the ILD test rest on the assumption that the slow loris clade is real and thus phylogenetically accurate. If this assumption is false, then the ILD test could be said to have performed nearly perfectly, giving accurate results when no heterogeneity was detected and false results when it was. Several lines of evidence suggest that this is not the case, however, and that our previous conclusions are justified. As enumerated in the introduction, the number and complexity of shared similarities among the slow lorises are impressive. Concluding that all of these characters arose through parallel evolution would call for a remarkable case of evolutionary convergence. Alternatively, fossil data appear to falsify the otherwise plausible hypothesis that slow loris characteristics are ancestral for the lorisiform clade. The fossil record of early lorisiform evolution supports the idea of a progressive morphological separation between two related clades (the galagos and lorises), both following highly divergent evolutionary pathways from a common, more generalized ancestor (Rasmussen and Nekaris, 1998).

Finally, several lines of evidence suggest that the molecular data are inconclusive at best with regard to slow loris phylogeny. Although the molecular data conflict with the monophyly hypothesis by suggesting paraphyly, the resolution of African and Asian slow lorises relative to the galagos is neither consistent nor robust. Taken alone, the molecular data show only poor resolution of the slow loris taxa rather than persuasive support for any particular topology. The strength of resolution as measured by the bootstrap and the branching order of a paraphyletic Loridae change, depending on which optimality criterion is used (likelihood or parsimony) and whether or not transversion weighting is applied in parsimony analysis. Moreover, the Kishino–Hasegawa test was unable to reject tree topologies in which the Loridae were constrained to be monophyletic. In fact, when the two data sets are analyzed under a maximum likelihood model partitioned by codon position, a tree with a monophyletic Loridae is chosen as most likely by the IRBP data (though not by the cytochrome *b* data). Taken as a whole, these results suggest that the apparent conflicts in the branching order of slow lorises are artifactual (e.g., sampling error attributable to insufficient molecular data) rather than actual. Indeed, this exercise in individual versus combined data analysis may have been most useful in focusing attention on the power of morphological data to resolve nodes that are weakly supported by molecular data. Such situations are likely to arise when cladogenesis is rapid but associated with strong adaptive divergence—events more likely to be recorded in the physical than in the genetic composition of an organism.

In summary, our study draws one of two conclusions. Either the ILD test is completely misleading as an indicator of the potential for phylogenetic accuracy in the combined data analyses, or, if the ILD results are to be believed and the slow loris clade is an artifact, these animals represent what must be among the most spectacular examples of parallel evolution in all of Eutheria. We consider the latter conclusion unlikely. Therefore, accepting the ILD results without further investigation could have led to a gross type I error by rejecting the null hypothesis of data homogeneity. For this reason, we recommend that the ILD never be used as a test of data partition combinability.

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REFERENCES

ALTERMAN, L. 1995. Toxins and toothcombs: Possible chemical defenses in Nycticebus and Perodicticus. Pages 413–424 *in* Creatures of the dark: The nocturnal prosimians (L. Alterman, G. A. Doyle, and M. K. Izard, eds.). Plenum, New York.

ANDERSON, S., A. T. BANKIER, B. G. BARRELL, M. H. L. DE BRUIJN, A. R. COULSON, J. DROUIN, I. C. EPERON, D. P. NIERLICH, B. A. ROE, F. SANGER, P. H. SCHREIER, A. J. H. SMITH, R. STADEN, AND I. G. YOUNG. 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457–465.

ANDREWS, T. D., L. S. JERMIIN, AND S. EASTEAL. 1998. Accelerated evolution of cytochrome *b* in simian primates: Adaptive evolution in concert with other mitochondrial proteins? J. Mol. Evol. 47:249–257.

BIBB, M. J., R. A. VAN ETTEN, C. T. WRIGHT, M. W. WALBERG, AND D. A. CLAYTON. 1981. Sequence and gene organization of mouse mitochondrial DNA. Cell 26:167–180.

BROWN, W. M., E. M. PRAGER, A. WANG, AND A. C. WILSON. 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. J. Mol. Evol. 18:225–239.

BULL, J. J., J. P. HUELSENBECK, C. W. CUNNINGHAM, D. L. SWOFFORD, and P. J. WADDELL. 1993. Partitioning and combining data in phylogenetic analysis. Syst. Biol. 42:384–397.

CAO, Y., J. ADACHI, AND M. HASEGAWA. 1998. Comment on the quartet puzzling method for finding maximum-likelihood tree topologies. Mol. Biol. Evol. 15:87–89.

CHARLES-DOMINIQUE, P. 1977. Ecology and behavior of nocturnal primates. Columbia Univ. Press, New York.

CHARLES-DOMINIQUE, P., AND R. D. MARTIN. 1970. Evolution of lorises and lemurs. Nature 227:257–260.

CHIPPINDALE, P. T., AND J. J. WIENS. 1994. Weighting, partitioning, and combining characters in phylogenetic analysis. Syst. Biol. 43:278–287.

CUNNINGHAM, C. W. 1997a. Is congruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. Syst. Biol. 46:464–478.

CUNNINGHAM, C. W. 1997b. Can three incongruence tests predict when data should be combined? Mol. Biol. Evol. 14:733–740.

DAVIS, J. I., M. P. SIMMONS, D. W. STEVENSON, AND J. F. WENDEL. 1998. Data decisiveness, data quality, and incongruence in phylogenetic analysis: an example from the monocotyledons using mitochondrial *atp* A sequences. Syst. Biol. 47:282–310.

DENE, H., M. GOODMAN, W. PRYCHODKO, AND G. W. MOORE. 1976. Immunodiffusion systematics of the primates: The Strepsirhini. Folia Primatol. 25:35–61.

DE QUEIROZ, A. 1993. For consensus (sometimes). Syst. Biol 42:368–372.

DESALLE, R., AND V. Z. BROWER. 1997. Process partitions, congruence, and the independence of characters: Inferring relationships among closely related Hawaiian Drospohila from multiple gene regions. Syst. Biol. 46:751–764.

DUTRILLAUX, B. 1988. Chromosome evolution in primates. Folia Primatol. 50:134–135.

DUTRILLAUX, B., AND Y. RUMPLER. 1995. Phylogenetic relations among Prosimii with special reference to Lemuriformes and Malagasy nocturnals Pages 141–156 *in* Creatures of the dark (L. Alterman, G. A. Doyle, and M. K. Izard, eds.). Plenum Press, New York.

EAGLEN, R. H. 1980. The systematics of living Strepsirhini, with special reference to the Lemuridae. Duke Univ., Durham, North Carolina.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. Cladistics 10:315–319.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1995. Constructing a significance test for incongruence. Syst. Biol. 44:570–572.

FELSENSTEIN, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27:401–410.

FLYNN, J. J., AND M. A. NEDBAL. 1998. Phylogeny of the Carnivora (Mammalia): congruence vs. incompatibility among multiple data sets. Mol. Phy. Evol. 9:414–426.

FONG, S. L., W. B. FONG, T. A. MORRIS, K. M. KEDZIE, AND C. D. BRIDGES. 1990. Characterization and comparative structural features of the gene for human interstitial retinol-binding protein. J. Biol. Chem. 265:3648–3653.

GOLOBOFF, P. 1991. Homoplasy and the choice among cladograms. Cladistics 7:215–232.

GOODMAN, M., C. A. PORTER, J. CZELUSNIAK, S. L. PAGE, H. SCHNEIDER, J. SHOSHANI, G. GUNNELL, AND C. P. GROVES. 1998. Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence. Mol. Phy. Evol. 9:585–598.

Gray, J. E. 1821. On the natural arrangement on vertebrose animals. London Medical Repository record 15:296–310.

GROVES, C. P. 1971. Systematics of the genus *Nycticebus*. Pages 44–53 *in* Proceedings of the Third International Congress of Primatology, Zurich, 1970, volume 1.

GROVES, C. P. 1993. Order primates. Pages 243–277 *in* Mammal species of the world: A taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, eds.). Smithsonian Institution Press, Washington, DC.

HILL, W. C. O. 1947. The lorisoid genus *Arctobecus*: Observations based on the type material. Proc. R. Soc. Edinb. 23:155–164.

HILLIS, D. M., AND J. J. BULL. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42:182–192.

HUELSENBECK, J. P., J. J. BULL, AND C. W. CUNNINGHAM. 1996. Combining data in phylogenetic analysis. Trends Ecol. Evol. 11:152–158.

HUELSENBECK, J. P., AND R. NEILSEN. 1999. Variation in the pattern of nucleotide substitution across sites. J. Mol. Evol. 48:86–93.

IKEDA, A., S. MIYAKE, K. IKEDA, AND S. HAYAMA. 1988. Three-dimensional analysis of the arterial pattern of the upper limbs in Lorisiformes (Prosimiae, Primates). Acta Anat. 132:297–303.

IKEDA, A., S. MIYAKE, AND S. MATSUMOTO (eds.). 1992. Arterial patterns in the upper limbs of Prosimii (Lorisidae, Galagidae, and Tarsiidae). Univ. of Tokyo Press, Tokyo.

JUKES, T. H. 1987. Transitions, transversions and the molecular clock. J. Mol. Evol. 26:87–98.

KISHINO, H., AND M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimates of the evolutionary tree topologies from sequence data, and the branching order in Hominoidea. J. Mol. Evol. 29:170–179.

LARSON, A. 1994. The comparison of morphological and moelcular data in phylogenetic systematics. Pages 371–390 *in* Molecular ecology and evolution: Approaches and applications (B. Schiewater, B. Streit, G. P. Wagner, and R. DeSalle, eds.). Birkhauser Verlag, Berlin.

MESSENGER, S. L., AND J. A. MCGUIRE. 1998. Morphology, molecules, and the phylogenetics of cetaceans. Syst. Biol. 47:90–124.

MICKEVICH, M. F., AND J. S. FARRIS. 1981. The implications of congruence in *Menidia*. Syst. Zool. 30:351–370.

MIYAMOTO, M. M. 1985. Consensus cladograms and general classifications. Cladistics 1:186–189.

MULLER, E. F., U. NIESCHALK, AND B. MEIER. 1985. Thermoregulation in the slender loris (*Loris tardigradus*). Folia Primatol. 44:216–226.

PORTER, C. A., S. L. PAGE, J. CZELUSNIAK, H. SCHNEIDER, M. P. C. SCHNEIDER, I. SAMPIO, AND M. GOODMAN. 1997. Phylogeny and evolution of selected primates as determined by sequences of the e-globin locus and 5′ flanking regions. Int. J. Primatol. 18:261–295.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14:817–818.

RASMUSSEN, D. T. 1986. Life history and behavior of slow lorises: Implications for the Lorisine–Galagine divergence. *In* Anthropology. Duke Univ., Durham, North Carolina.

RASMUSSEN, D. T., AND M. K. IZARD. 1988. Scaling of life history traits relative to body size, brain size and metabolic rate in lorises and galagos (Lorisidae, Primates). Am. J. Phys. Anthropol. 75:357–367.

RASMUSSEN, D. T., AND K. A. NEKARIS. 1998. Evolutionary history of lorisiform primates. Folia Primatol. 69:250–285.

RODRIGO, A. G., M. KELLEY-BORGES, P. R. BERGQUIST, AND P. L. BERGQUIST. 1993. A randomization test of the null hypothesis that two cladograms are sample estimates of a parametric phylogenetic tree. N. Z. J. Bot. 31:257–268.

RUMPLER, Y., S. WARTER, B. MEIER, H. PREUSCHOFT, AND B. DUTRILLAUX. 1987. Chromosomal phylogeny of three lorisidae: *Loris tardigradus*, *Nycticebus coucang*, and *Perodicticus potto*. Folia Primatol. 48:216–220.

RUNESTAD, J. A. 1997. Postcranial adaptations for climbing in Loridae (Primates). J. Zool. 242:261–290.

SANDERSON, M. J., AND M. J. DONOGHUE. 1989. Patterns of variation in levels of homoplasy. Evolution 43:1781–1795.

SARICH, V. M., AND J. E. CRONIN. 1976. Molecular systematics of the primates. Pages 141–170 *in* Molecular anthropology (M. Goodman and R. E. Tashian, eds.). Plenum, New York.

SCHWARTZ, J. H. 1992. Phylogenetic relationships of African and Asian lorisids. Pages 103–112 *in* Topics in primatology: Evolutionary biology, reproductive endrocrinology, and virology (S. Matano, R. H. Tuttle, H. Ishida, and M. Goodman, eds.). Univ. of Tokyo Press, Tokyo.

SIDALL, M. E. 1997. Prior agreement: Arbitration or arbitrary? Syst. Biol. 46:765–769.

STANHOPE, M. J., J. CZELUSNIAK, J.-S. SI, J. NICKERSON, AND M. GOODMAN. 1992. A molecular perspective on mammalian evolution from the gene encoding interphotoreceptor retinoid binding protein, with convincing evidence for bat monophyly. Mol. Phylogenet. Evol. 1:148–160.

STANHOPE, M. J., M. R. SMITH, V. G. WADDELL, C. A. PORTER, M. S. SHIVJI, AND M. GOODMAN. 1996. Mammalian evolution and the interphotoreceptor retinoid binding protein (IRBP) gene: Convincing evidence for several superordinal clades. J. Mol. Evol. 43:83–92.

STRIMMER, K., N. GOLDMAN, AND A. VON HAESELER. 1997. Bayesian probabilities and quartet puzzling. Mol. Biol. Evol. 14:210–211.

STRIMMER, K., AND A. VON HAESLER. 1996. Quartet puzzling: A quartet maximum-likelihood method for reconstructing tree topologies. Mol. Biol. Evol. 13:964–969.

SULLIVAN, J. 1996. Combining data with different distributions of among-site variation. Syst. Biol. 45:375–380.

SWOFFORD, D. L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods), version 4.0b1. Sinauer Associates, Sunderland, Massachusetts.

SZALAY, F. S., AND C. C. KATZ. 1973. Phylogeny of lemurs, galagos and lorises. Folia Primatol. 19:88–103.

TAMURA, K., AND M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512–526.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37:221–244.

THORNTON, J. W., AND R. DESALLE. 2000. A new method to localize and test the significance of incongruence: Detecting domain shuffling in the nuclear receptor superfamily. Syst. Biol. 49:183–201.

VOELKER, G., AND S. V. EDWARDS. 1998. Can weighting improve bushy trees? Models of cytochrome *b* evolution and the molecular systematics of pipits and wagtails (Aves: Motacillidae). Syst. Biol. 47:589–603.

WAKELEY, J. 1996. The excess of transitions among nucleotide substitutions: New methods of estimating transition bias underscore its significance. Trends Ecol. Evol. 11:158–163.

WHITTOW, G. C., C. A. SCAMMELL, J. K. MANUEL, D. RAND, AND M. LEONG. 1977. Temperature regulation in a hypometabolic primate, the slow loris (*Nycticebus coucang*). Arch. Int. Physiol. Biochim. 85:139–151.

WIENS, J. J. 1998. Combining data sets with different phylogenetic histories. Syst. Biol. 47:568–581.

WISLOCKI, G. B., AND W. L. STRAUS. 1932. On the blood vascular bundles in the limbs of certain edentates and lemurs. Bull. Mus. Comp. Zool. 74:1–15.

YANG, Z., AND A. D. YODER. 1999. Estimation of the transition/transversion rate bias and species sampling. J. Mol. Evol. 48:274–283.

YODER, A. D. 1992. The phylogenetic affinities of the Cheirogaleidae: A molecular and morphological analysis. *In* Anatomy. Duke Univ., Durham, North Carolina.

YODER A. D. 1994. Relative position of the Cheirogaleidae in strepsirhine phylogeny: A comparison of morphological and molecular methods and results. Am. J. Phys. Anthropol. 94:25–46.

YODER, A. D., AND J. A. IRWIN. 1999. Phylogeny of the Lemuridae: Effects of character and taxon sampling on resolution of species relationships within *Eulemur*. Cladistics 15:351–361.

YODER, A. D., M. RUVOLO, AND R. VILGALYS. 1996. Molecular evolutionary dynamics of cytochrome *b* in strepsirrhine primates: The phylogenetic significance of third position transversions. Mol. Biol. Evol. 13:1339–1350.

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