Syst. Biol. 52(6):839–848, 2003
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ISSN: 1063-5157 print / 1076-836X online
DOI: 10.1080/10635150390252242

Novel Versus Unsupported Clades: Assessing the Qualitative Support for Clades in MRP Supertrees

OLAF R. P. BININDA-EMONDS

Lehrstuhl für Tierzucht, Technical University of Munich, Alte Akademie 12, 85354 Freising–Weihenstephan, Germany;
E-mail: Olaf.Bininda@tierzucht.tum.de

Abstract.— Matrix representation with parsimony (MRP) supertree construction has been criticized because the supertree may specify clades that are contradicted by every source tree contributing to it. Such unsupported clades may also occur using other supertree methods; however, their incidence is largely unknown. In this study, I investigated the frequency of unsupported clades in both simulated and empirical MRP supertrees. Here, I propose a new index, *QS*, to quantify the qualitative support for a supertree and its clades among the set of source trees. Results show that unsupported clades are very rare in MRP supertrees, occurring most often when there are few source trees that all possess the same set of taxa. However, even under these conditions the frequency of unsupported clades was <0.2%. Unsupported clades were absent from both the Carnivora and Lagomorpha supertrees, reflecting the use of large numbers of source trees for both. The proposed *QS* indices are correlated broadly with another measure of quantitative clade support (bootstrap frequencies, as derived from resampling of the MRP matrix) but appear to be more sensitive. More importantly, they sample at the level of the source trees and thus, unlike the bootstrap, are suitable for summarizing the support of MRP supertree clades. [Carnivora; conflict; Lagomorpha; matrix representation; MRP; phylogenetic supertrees; simulation; support.]

Supertree construction involves the combination of many source trees to yield a single, usually more inclusive, supertree. Because the source trees need only be partially overlapping, the supertree may specify clades that allow novel statements of relationship between taxa that are not present simultaneously in any single source tree (Sanderson et al., 1998). This is a feature shared by all supertree methods and is one defining characteristic of the supertree approach.

In some supertree methods, however, it is possible that some new combinations of taxa do not possess even implied support within the set of source trees as a whole. Instead, these combinations are contradicted by every source tree. I previously referred to these clades as simply "novel clades" (Bininda-Emonds and Bryant, 1998), which unfortunately is slightly imprecise in light of the Sanderson et al. (1998) usage above. To reduce confusion, I will refer to both supported (sensu Sanderson et al., 1998) and unsupported novel clades (sensu Bininda-Emonds and Bryant, 1998) or, more simply, supported and unsupported clades.

Only supertree methods that reanalyze representations of the source trees have the potential to yield unsupported clades. Such methods are largely indirect (sensu Wilkinson et al., 2001) and include matrix representation with parsimony (MRP; Baum, 1992; Ragan, 1992), MinCutSupertrees (Semple and Steel, 2000), MinFlip supertrees (Chen et al., 2001), and average consensus (Lapointe and Cucumel, 1997). Other largely direct supertree methods that simply combine source trees and output common or uncontradicted components cannot generate unsupported clades. In the case of MRP, the appearance of unsupported clades has been likened to the phenomenon of signal enhancement (sensu de Queiroz et al., 1995) in total evidence analyses (Bininda-Emonds et al., 1999; Bininda-Emonds and Sanderson, 2001). However, this viewpoint has been criticized strongly by Pisani and colleagues (Pisani and Wilkinson, 2002; Pisani et al., 2002; Wilkinson et al., 2004), who argued that any unsupported clades are just that and should be collapsed because they have no evidential basis given the data. In any case, the incidence of unsupported clades in supertree analysis is largely unknown. The only figures come from Bininda-Emonds and Bryant (1998), who indicated that about 4% of the 198 clades in an MRP supertree of the Carnivora (Bininda-Emonds et al., 1999) were not present among the source trees, and Pisani et al. (2002), who indicated that no clades in the Dinosauria MRP supertree were unsupported.

In this study, I defined and then examined qualitative support for clades in MRP supertrees using both simulation and a pair of empirical examples: the supertrees of the mammalian orders Carnivora (Bininda-Emonds et al., 1999) and Lagomorpha (Stoner et al., 2003). Further, I examined the relationship between the qualitative support for a supertree clade and its size and quantitative support (as measured by nonparametric bootstrap frequencies) to determine whether unsupported clades possess common characteristics or can be identified by proxy measures. I focused exclusively on MRP because it is by far the most popular supertree method and the only one that has attracted any serious criticism in the current context. Here, I propose the *QS* index to measure the qualitative support for the clades in a supertree and for the supertree as a whole. This index can be applied to a supertree obtained using any method (or even any two trees with different taxon sets) but is perhaps the most relevant for matrix representation methods. In the latter case, the *QS* index is one of the first support measures that is unaffected by the inherent nonindependence of the coding method used in matrix representation (see Purvis, 1995).

MATERIALS AND METHODS

Qualitative Support: Defining Properties of Clades

Many measures exist to compare trees with identical taxon sets (for a partial list, see Swofford, 1991). However, an inherent difficulty exists in comparing trees with overlapping taxon sets. Absolute assessments of support or conflict are often prohibited by the presence of missing taxa, which may or may not contradict a given statement of relationship depending on where they are placed in the tree. Therefore, it is necessary to refer to hard and soft forms of support and conflict (or analogous terms) when referring to qualitative support for supertree clades. Determining whether or not a given clade on a supertree is unsupported requires three nested levels of comparison: (1) with a single clade on a source tree (2) with the set of clades on a source tree, and (3) with the set of source trees.

The first level of comparison is the most critical but also the most subjective. At this level, a given clade on a source tree may show a hard match or a hard mismatch or may be equivocal with respect to a clade on a supertree (Table 1). I define a hard match as the case when the supertree clade is specified exactly. A hard match therefore requires that both trees possess the same taxon sets. A hard mismatch occurs when the source tree clade contradicts directly the relationships presented in the supertree clade. As such, all the taxa in the supertree clade must be present in the source tree. Equivocal matches usually result from the presence of missing taxa in the source tree and can be broken down further into soft matches, soft mismatches, and (true) equivocalness. In a soft match, addition of the missing taxa may support the supertree clade but never contradict it. Conversely, in a soft mismatch, the missing taxa may contradict the supertree clade but never support it. True equivocal matches result when the supertree clade contains the source tree clade or vice versa or when the missing taxa can both support and contradict the supertree clade.

These concepts can also be viewed in terms of pairwise compatibility between the representations of the supertree and source tree clades. As shown in Table 1, each clade can be described by a set of characters (e.g. supertree clade: 111000; source tree 1 clade: 110000). If the supertree and source tree clades are compared position by position, four pairwise patterns are possible excluding missing data (00, 01, 10, and 11). For two clades to be compatible, no more than three of the four possible patterns can be present. This is the case for the example cited, where there are only three patterns (11, 10, and 00). This is also true for hard, soft, and equivocal matches (when missing data have been accounted for). For hard mismatches, all four patterns are present and the representations are pairwise incompatible. For soft mismatches, either three or four patterns can be present depending on how the missing data are resolved. Thus, the

TABLE 1. The first level of comparison between a clade in a supertree and a single clade in a source tree. The supertree clade consists of taxa A, B, and C to the exclusion of D, E, and F: ((A, B, C) D, E, F). The source tree clades differ in the placement or presence of taxa C and D (underlined). Support for the supertree clade given by each source tree is indicated, with alternative outcomes for the different placements of missing taxa when applicable. The interpretation of support is determined by whether or not the source tree clade does or does not support (i.e., is compatible with) or contradict (i.e., is incompatible with) the supertree clade.

	Taxa						Possible placement of missing taxa						
Clade	A	B	C	D	E	F	Best case	Worst case	Supports	Does not support	Contradicts	Does not contradict	Interpretation
Supertree	1	1	1	0	0	0							
Source 1	1	1	0	0	0	0				x		x	Equivocal; supertree contains source tree clade
Source 2	1	1	0	1	0	0				x	x		Hard mismatch
Source 3	1	1	0	?	0	0	Equivocal; supertree contains source tree clade (0)	Mismatch (1)		x	x	x	Soft mismatch
Source 4	1	1	1	0	0	0			x			x	Hard match
Source 5	1	1	1	1	0	0				x		x	Equivocal; source tree clade contains supertree clade
Source 6	1	1	1	?	0	0	Hard match (0)	Equivocal; source tree clade contains supertree clade (0)	x	x		x	Soft match
Source 7	1	1	?	0	0	0	Hard match (1)	Equivocal; supertree contains source tree clade (0)	x	x		x	Soft match
Source 8	1	1	?	1	0	0	Equivocal; source tree clade contains supertree clade (1)	Hard mismatch (0)		x	x	x	Soft mismatch
Source 9	1	1	?	?	0	0	Hard match (1, 0)	Hard mismatch (0, 1)	x	x	x	x	Equivocal

concepts of supports, does not support, contradicts, and does not contradict from Table 1 can also be viewed in terms of compatible, not compatible, incompatible, and not incompatible, respectively.

At the second comparison level, the results from the first comparison level are summed over all the clades in the source tree. If at least one source tree clade provides a hard match or hard mismatch to the supertree clade, then so does the source tree as a whole. Otherwise, the source tree is equivocal with respect to the supertree clade (i.e., it is neither supported nor contradicted directly). Again, it is possible to define soft matches and mismatches. For a soft match, the missing taxa will never contradict the clade (i.e., number of individual soft matches > number individual soft mismatches = 0), whereas for a soft mismatch they will never support it (i.e., number of individual soft mismatches > number individual soft matches = 0). True equivocalness represents all remaining options.

At the third comparison level, the results from the second comparison level are summed over the set of source trees to indicate the qualitative support for the supertree clade in the form of hard or soft support, hard or soft conflict, or equivocal support. I define hard support as the case where the supertree clade is specified exactly in at least one source tree (i.e., number of hard matches ≥ 1). Hard support will necessarily be rare in the supertree setting given that it requires at least one source tree to have the same taxon set as the supertree. Hard conflict occurs when the supertree clade is contradicted by every source tree (i.e., is unsupported; number of hard mismatches = number of source trees). A supertree clade has soft support when it is not specified exactly by any source tree but not contradicted by any source tree either (i.e., number of hard matches = number of hard mismatches = 0). Soft conflict is defined as when the supertree clade is contradicted by at least one but not all source trees (i.e., 0 < number of hard mismatches < number of source trees). Equivocal support occurs when all source trees are equivocal with respect to the supertree clade (i.e., number of equivocal matches = number of source trees).

An alternative at the first level of comparison is to eliminate the categories of soft matches and mismatches by pruning the supertree down to the taxon set found in the source tree. However, doing so will inflate the number of hard matches. In Table 1, both instances of a soft match (for source tree clades 6 and 7) would become hard matches, as would the equivocal case with clade 9. In contrast, both instances of soft mismatches (clades 3 and 8) would become equivocal. Thus, the procedure as stated above represents a more conservative estimate of the qualitative support for a supertree.

Quantifying Support on the Supertree

It is possible to derive an index to quantify the qualitative support (*QS*) for a supertree clade among the set of source trees. At the second level of comparison, hard matches between a given supertree clade and each source tree are scored as +1, soft matches as +0.5, equivocal matches as 0, soft mismatches as −0.5, and hard mismatches as −1. For each supertree clade, these values are summed over all source trees and divided by the number of source trees. Thus, QS_{clade} varies between −1 and +1, with the two values indicating that the supertree clade possesses hard mismatches and hard matches, respectively, with every source tree in the set. In general, negative values of QS_{clade} indicate that a supertree clade shows proportionately more or stronger mismatches than matches within the set of source trees, whereas positive values indicate the reverse. It is possible for a supertree clade with hard support to possess a $QS_{\mathrm{clade}} < 0$ because hard support requires a hard match with only a single source tree. One can also calculate the quantitative support for the supertree as a whole (QS_{tree}) as the average QS_{clade} value among all the clades in a supertree.

Values of QS_{clade} and QS_{tree} are expected to decrease as the number of source trees and/or the size of the supertree clade increase, because both factors increase the probability of conflict (Bininda-Emonds and Sanderson, 2001). Similarly, values will be lower in the supertree setting (compared with the consensus tree setting) and with an increased number of smaller source trees, because both factors will result in proportionately fewer hard matches. In each case, however, QS_{clade} still seems to provide an accurate reflection of the amount of conflict within the set of source trees.

Simulation Protocol

In addition to two empirical examples, I used simulation to investigate the frequency of unsupported clades in MRP supertrees. The simulation protocol is similar to the one that I have used elsewhere to elucidate the behavior of MRP supertree construction (Bininda-Emonds and Sanderson, 2001; Bininda-Emonds, 2003). A Yule branching process was used to construct a known model tree using r8s (Sanderson, 2003). Branch lengths were modeled to depart from the assumption of a molecular clock (see Bininda-Emonds and Sanderson, 2001). Nucleotide sequences were then evolved along the model tree according to a standard Markov model using Seq-Gen 1.1 (Rambaut and Grassly, 1997). The resultant data set was subdivided into equal partitions of 500 nucleotides each according to the desired number of source trees (for specific details concerning the preceding steps, see Bininda-Emonds and Sanderson, 2001). Each partition was analyzed individually using parsimony (PAUP* 4.0b10; Swofford, 2002) to produce individual source trees. The set of source trees were then coded using standard MRP (using the Perl script SuperMRP.pl), with the resulting matrix analyzed using PAUP* to yield the MRP supertree. Values of both QS_{clade} and QS_{tree} were derived using the Perl script QualiTree.pl.

I quantified evidential support for the clades in the supertree using the bootstrap (Felsenstein, 1985), with the individual elements of the MRP matrix being resampled. I did this largely as a heuristic to obtain a rough indication of support. MRP uses additive binary coding to encode any given source tree. For each node in turn, taxa that are descended from it are scored as 1, whereas taxa that are not are scored as 0 unless they are missing from that source tree, in which case they receive a question mark. Thus, given the hierarchical structure of a tree, the MRP coding of it is inherently nonindependent, which violates the boostrap's assumption that the characters are independent and identically distributed (IID; Felsenstein, 1985; Purvis, 1995). Thus, my use of the bootstrap is invalid, strictly speaking. However, the same nonindependence problem probably also affects other character-based support measures such as Bremer's decay index (Bremer, 1988; Källersjö et al., 1992). Bremer support also has the disadvantage in that, unlike bootstrap values, the values are not standardized and therefore not easily comparable between or even within studies (DeBry, 2001). In any case, given that all source trees are about equal in size and resolution, no single source tree will be dominant and the nonindependence will be more equally distributed.

Parameters for the simulation runs were: number of taxa (5, 20 or 50), number of source trees (20 or 50), deletion probability for a given species to yield source trees with different taxon sets (0, 0.1, or 0.5), and rate of evolution in Seq-Gen (homoplasy level, 0.1). All combinations of these parameters were examined, with 500 replicates per run. I refer to the case when all trees had identical versus partially overlapping taxon sets (i.e., a deletion probability of 0 vs. >0, respectively) as the consensus and supertree settings, respectively (following Bininda-Emonds et al., 2002). In comparing values of QS_{clade} with bootstrap frequencies for the clades on a supertree, the parameters for the simulation runs were identical except that only 20 source trees and only 50 replicates were used.

I employed a simplified search strategy to derive the source trees because their accuracy was not important given that no comparisons to the model tree were being made. Heuristic searches used the closest addition sequence and saved only a single tree; characters were still weighted to correspond with the simulated model of molecular evolution. Parsimony searches of the MRP matrix to derive the supertree used the parsimony ratchet (Nixon, 1999), an iterative approach employing data reweighting to search tree space. For each iteration, the starting point for branch swapping is the tree from the immediately preceding iteration. However, the search is done with a given proportion of randomly selected characters reweighted. Searches employ a highly simplified and fast strategy, with only single trees being retained. Altogether, it is thought that the efficiency of the ratchet derives from it being able to sample quickly from many tree islands rather than exhaustively searching any single island (Nixon, 1999).

Ratchet analyses were run in PAUP* using a command block generated by the Perl script PerlRat.pl. Parameters for the ratchet were a character deletion probability of 0.25, 1,000 iterations, and no terminal "brute force" search on the trees retained from the iterations. The supertree was held to be the strict consensus of all trees from the ratchet iterations with the same minimum length (maximum $n = 1{,}000$). Finally, the bootstrap analysis employed the PAUP* "faststep" search algorithm (i.e., heuristic search with a single random addition sequence and no branch swapping, saving a single tree; see Swofford, 2002) with 10,000 replicates. Altogether, this strategy allowed the examination of the behavior of MRP with more taxa and source trees than in my previous simulation studies.

RESULTS

Simulated Data

Consensus setting.—When the source trees all possessed identical taxon sets, virtually every supertree clade possessed hard support (>99.92% of all 96,245 clades over all replicates and simulation parameters). This was true regardless of the size or number of source trees, or the size or inferred bootstrap support of the supertree clade (Table 2). Average bootstrap frequencies were high on average (>91%) for these supertree clades. The high positive values for QS_{tree} (minimum average value of 0.631) also indicated that the supertree clades were supported by the majority of the source trees.

Over all simulation runs, only 70 clades showed hard conflict among the source trees; 56 of these clades resulted when there were many taxa (50) and few source trees (5). There were also two independent cases of a clade with equivocal support. All the unsupported and equivocal clades demonstrated poor quantitative support (average bootstrap frequency <35%) and, except for the two with equivocal support, tended to be relatively large, containing from 33% to 56% of the taxa on the supertree.

Supertree setting.—Regardless of the completeness of the source trees, supertree clades were virtually never contradicted by every source tree (Table 3). Over all simulation runs, only 61 of 186,072 clades (<0.03%) possessed hard conflict. Again, the majority of these cases occurred under one set of conditions (5 source trees, 50 taxa, and a 10% deletion probability), but they still constituted only 0.21% of all clades for that set of conditions. All unsupported clades were poorly supported (average bootstrap frequency <23%) and relatively large, containing from 15% to 66% of all the taxa on the supertree.

Compared with the consensus setting, far fewer supertree clades possessed hard support. Hard support was present only when the source trees were highly overlapping (i.e., contained about 90% of all taxa on average) and noticeably more so when they had up to 20 as opposed to 50 taxa. Instead, supertree clades possessed qualitative support in categories that were virtually absent in the consensus setting: soft or equivocal support, or soft conflict. In general, the frequency of soft conflict increased with an increase in either source tree size or number; however, the frequency was never >50% of the clades in a set of replicates. Supertree clades displaying soft conflict were also noticeably larger in size

TABLE 2. Qualitative support for the clades in an MRP supertree relative to the source trees. Data were simulated according to the procedure in the text. Standard deviations appear in parentheses below each mean ($n = 500$ replicates per set of simulation parameters). Source trees had identical taxon sets (consensus setting).

No.				Hard support			Soft support			Hard conflict			Soft conflict			Equivocal		
source trees	No. taxa	No. clades	QS_{tree}	No. clades	Bootstrap freq.	Clade size	No. clades	Bootstrap freq.	Clade size	No. clades	Bootstrap freq.	Clade size	No. clades	Bootstrap freq.	Clade size	No. clades	Bootstrap freq.	Clade size
5	20	17.0	0.789	17.0	93.5	6.0	0			0.0	29.7	7.7	0			0		
		(1.0)	(0.091)	(1.1)	(3.1)	(0.7)				(0.1)	(9.1)	(3.2)						
5	50	45.0	0.722	44.8	91.9	8.2	0			0.1	29.7	18.0	0.0	52.1	17.5	0.0	31.6	2.0
		(1.9)	(0.075)	(1.9)	(2.3)	(1.1)				(0.4)	(15.1)	(11.7)	(0.1)	(16.3)	(9.2)	(0.0)		
20	20	17.8	0.711	17.8	96.4	6.0	0			0.0	24.1	9.5	0			0		
		(0.5)	(0.116)	(0.5)	(2.9)	(0.7)				(0.1)	(3.9)	(6.4)						
20	50	47.1	0.650	47.1	95.5	8.3	0			0.0	30.3	16.6	0			0.0	33.8	2.0
		(1.0)	(0.075)	(1.0)	(2.0)	(1.1)				(0.1)	(18.5)	(13.5)				(0.0)		
50	20	17.9	0.703	17.9	97.4	6.0	0			0			0			0		
		(0.3)	(0.109)	(0.3)	(2.5)	(0.7)												
50	50	47.7	0.631	47.7	97.0	8.4	0			0.0	24.3	28.0	0			0		
		(0.6)	(0.080)	(0.6)	(1.6)	(1.0)				(0.1)	(1.4)	(5.7)						

TABLE 3. Qualitative support for the clades in an MRP supertree relative to the source trees. Data were simulated according to the procedure in the text. Standard deviations appear in parentheses below each mean (n = 500 replicates per set of simulation parameters). Source trees had partially overlapping taxon sets ("supertree setting"), with either 10% or 50% of taxa deleted at random from each source tree.

				Hard support			Soft support			Hard conflict			Soft conflict			Equivocal		
No. source trees	No. taxa	No. clades	QS_{tree}	No. clades	Bootstrap freq.	Clade size	No. clades	Bootstrap freq.	Clade size	No. clades	Bootstrap freq.	Clade size	No. clades	Bootstrap freq.	Clade size	No. clades	Bootstrap freq.	Clade size
10% taxa deleted																		
5	20	16.8	0.063	7.7	93.9	6.1	3.0	93.5	4.8	0.0	18.5	11.0	3.1	70.7	6.4	3.0	96.5	7.2
		(1.2)	(0.151)	(7.8)	(2.8)	(0.8)	(3.1)	(9.4)	(1.1)	(0.1)	(8.4)	(1.4)	(2.4)	(11.7)	(3.0)	(3.2)	(3.9)	(2.1)
5	50	44.6	−0.118	0.9	92.2	8.0	13.9	95.7	5.4	0.1	22.9	21.6	14.8	71.9	10.1	14.9	97.6	9.4
		(2.1)	(0.054)	(5.9)	(2.7)	(0.7)	(3.0)	(2.6)	(0.7)	(0.4)	(11.8)	(10.3)	(3.8)	(5.8)	(3.1)	(3.8)	(1.3)	(2.8)
20	20	17.7	0.026	16.0	96.8	6.0	0.2	98.2	4.8	0			1.3	76.3	6.2	0.2	100.0	7.4
		(0.6)	(0.094)	(3.8)	(2.6)	(0.8)	(1.1)	(6.3)	(2.2)				(2.2)	(17.9)	(3.7)	(0.9)	(0.0)	(2.3)
20	50	47.1	−0.154	4.2	97.6	7.8	11.2	99.8	5.9	0.0	27.0	14.3	22.7	88.5	9.3	8.9	100.0	9.6
		(1.1)	(0.041)	(12.2)	(1.5)	(1.5)	(4.4)	(1.8)	(1.0)	(0.1)	(25.8)	(10.2)	(6.0)	(5.8)	(2.2)	(3.9)	(0.0)	(3.9)
50	20	17.9	0.017	17.6	97.5	6.0	0.0	100.0	4.8	0			0.2	71.3	6.1	0.0	100.0	9.3
		(0.4)	(0.073)	(1.1)	(2.2)	(0.7)	(0.1)	(0.0)	(4.0)				(0.8)	(18.9)	(4.2)	(0.1)		
50	50	47.6	−0.163	9.5	98.7	8.1	8.6	100.0	6.4	0			23.7	91.9	9.1	5.9	100.0	10.4
		(0.7)	(0.039)	(17.1)	(1.0)	(1.3)	(5.2)	(0.0)	(1.2)				(9.4)	(6.1)	(2.6)	(3.8)	(0.0)	(4.5)
50% taxa deleted																		
5	20	14.7	−0.252	0			5.7	59.3	5.0	0			1.6	24.7	8.3	7.4	35.3	7.0
		2.6	(0.056)				(1.3)	(8.3)	(0.8)				(1.4)	(14.7)	(3.3)	(2.2)	(12.5)	(1.7)
5	50	39.1	−0.318	0			13.7	56.4	5.4	0.0	1.6	23.8	7.2	19.6	14.0	18.3	34.7	8.9
		4.4	(0.037)				(1.9)	(5.4)	(0.5)	(0.1)	(4.2)	(10.0)	(3.1)	(8.9)	(4.9)	(3.9)	(7.8)	(2.5)
20	20	17.1	−0.306	0			6.1	85.9	4.9	0			5.9	63.8	6.9	5.0	84.9	6.9
		(1.1)	(0.030)				(1.2)	(6.4)	(0.6)				(1.8)	(10.6)	(1.8)	(1.7)	(7.2)	(2.2)
20	50	45.2	−0.369	0			14.2	84.0	5.4	0.0	7.3	18.0	18.4	61.5	10.9	12.5	84.7	8.5
		(2.0)	(0.021)				(2.0)	(4.5)	(0.5)	(0.0)			(3.3)	(6.9)	(2.4)	(2.7)	(4.8)	(3.1)
50	20	17.6	−0.313	0			5.6	98.6	5.3	0			8.0	82.6	6.1	4.1	98.7	7.3
		(0.7)	(0.026)				(1.4)	(1.6)	(1.1)				(1.8)	(8.1)	(1.4)	(1.6)	(0.9)	(2.8)
50	50	46.8	−0.375	0			12.9	98.4	5.8	0			24.5	81.8	9.7	9.4	98.9	9.2
		(1.2)	(0.019)				(2.3)	(1.2)	(0.7)				(3.0)	(5.3)	(1.7)	(2.2)	(0.6)	(3.7)

and possessed poorer bootstrap support (usually 20% lower) than clades with soft or equivocal support, especially when the source trees were highly nonoverlapping. Clades with soft support never constituted >33% of the clades in a set of replicates, with the frequency decreasing with increasing source tree number and especially size. These clades also always contained fewer than seven species on average, regardless of the size of the source trees. Clades with equivocal support showed largely the same trends as those with soft support, although they constituted about 50% of all clades when source trees were few (5) and highly nonoverlapping (50% deletion probability).

Behavior of QS indices.—As mentioned, values of QS_{tree} were high and positive under the consensus setting on average. These values decreased markedly in the supertree setting (<0.026) and as source trees became increasingly nonoverlapping: −0.1 to 0 with high overlap compared with about −0.3 with low overlap. In all cases, values of QS_{tree} decreased with both increasing source tree number and especially size. The decreases were especially great when source trees were highly overlapping, possibly because of the large difference in the presence of clades with hard support between source trees of different size under these conditions.

For individual clades, values of QS_{clade} correlated broadly with the bootstrap support for that clade (Fig. 1). The correlation coefficient over a total of 7,734 clades was 0.542 ($Z = 53.4$, $P < 0.0001$) and usually much higher when the values were split according to the number of source trees and their degree of overlap. The correlation generally worsened as source tree number increased and overlap decreased, but was always highly significant. QS_{clade} is more conservative than the bootstrap, typically assigning lower analogous values. This may be due in part to it appearing to be more sensitive than the bootstrap. For example, clades with 100% bootstrap support usually display a range of values of QS_{clade}, including negative values, which indicate greater conflict than support for that node.

Empirical Examples

As with the simulated data, instances of unsupported clades demonstrating hard conflict were extremely rare and, in fact, completely absent in both the Carnivora and Lagomorpha supertrees (Table 4). Somewhat surprising was the presence of clades with hard support, particularly at the very high frequencies observed in the Carnivora supertree. However, many of these instances of hard support derive from the inclusion of taxonomies, which typically include all species, as source trees. This is particularly true for the Lagomorpha

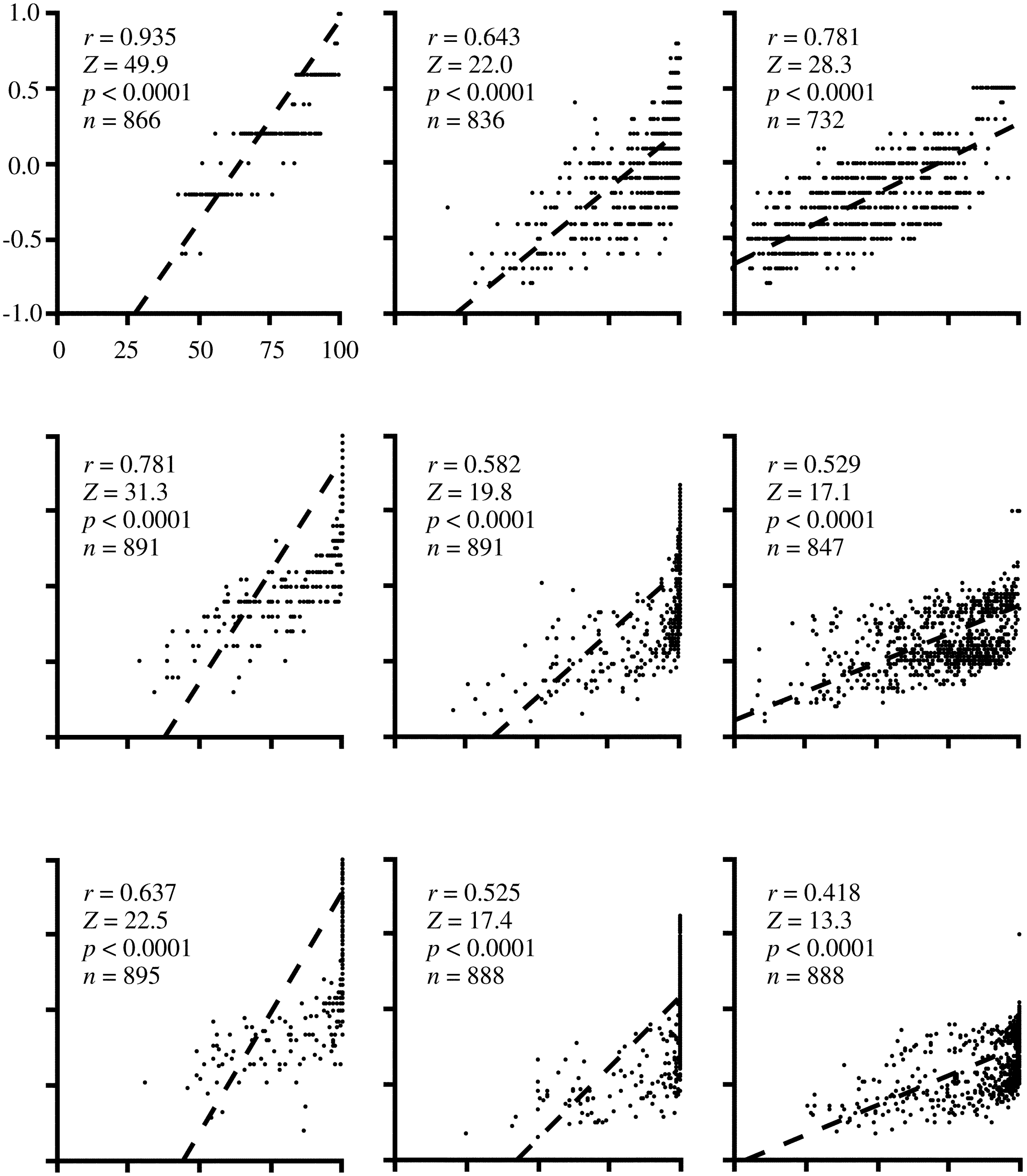

FIGURE 1. Comparison of QS_{clade} (y-axis) and bootstrap frequencies (x-axis) for clades in a supertree across a range of simulation parameters. Rows from top to bottom represent runs with 5, 20, and 50 source trees; columns from left to right represent runs with a deletion probability of 0%, 10%, and 50%. All graphs have the same scale (given in the top left graph only). Correlation statistics are provided for each individual graph. The linear regression line is provided for reference only.

supertree, where clades with hard support are comparatively large (15.9 species on average), reflecting the large number of species in most genera. Clades with hard support were also more prevalent in the smaller carnivore supertrees (i.e., those with <20 taxa) presumably because of the increased likelihood for some source trees, whether taxonomies or not, to include all the species in the group. One exception is the Felidae supertree, where the high number of clades with hard support is also surprising, given how little agreement there has been among felid phylogenies (Bininda-Emonds et al., 1999).

TABLE 4. Qualitative support for the clades in two empirical MRP supertrees relative to the source trees. The supertree for the Carnivora (Bininda-Emonds et al., 1999) is also presented as its separate hierarchically nested supertrees. The supertree for the Lagomorpha (Stoner et al., 2003: fig. 2) is the weighted version preferred by those authors. Standardized support values were not available for either supertree.

			Hard support		Soft support		Hard conflict		Soft conflict		Equivocal	
Taxon	Total no. clades	QS_{tree}	No. clades	Mean size	No. clades	Mean size	No. clades	Mean size	No. clades	Mean size	No. clades	Mean size
Lagomorpha	76	−0.109	11	14.6	4	2.3	0		61	9.6	0	
Carnivora	198	−0.029	132	5.1	10	2.3	0		52	8.4	4	4.6
Canidae	22	−0.146	8	4.8	0		0		14	7.4	0	
Higher groups	10	−0.206	7	4.0	0		0		3	3.7	0	
Felidae	33	−0.219	26	5.1	0		0		7	16.3	0	
Herpestidae	19	−0.158	9	7.0	2	2.0	0		7	12.1	1	4.0
Hyaenidae	1	0.500	1	3.0	0		0		0		0	
Lutrinae	8	0.083	8	3.1	0		0		0		0	
Mephitinae	6	0.250	5	2.8	0		0		1	4.0	0	
Mustelidae	31	−0.143	9	11.4	8	2.9	0		13	7.3	1	5.0
Otariidae	8	0.025	7	4.4	0		0		0		1	7.0
Phocidae	16	−0.085	16	4.7	0		0		0		0	
Procyonidae	8	−0.089	6	5.7	0		0		1	10.0	1	3.0
Ursidae	5	−0.029	5	4.0	0		0		0		0	
Viverridae	31	−0.045	25	6.5	0		0		6	4.3	0	

The only other category with a sizable proportion of clades was that of soft conflict, meaning that at least one but not all of the source trees contradicted the supertree clade. On average, clades demonstrating soft conflict contained more taxa than those showing either hard or soft support.

The utility of the qualitative support categories and QS_{clade} is demonstrated in Figure 2. Although most clades of the top-level supertree of the Carnivora (Bininda-Emonds et al., 1999) are supported directly in at least one source tree (hard support), the many negative values of QS_{clade} indicate that there is disagreement among the source trees. Three clades possess soft conflict, which arises in part from the highly unstable position of the red panda (*Ailurus fulgens*) in the supertree (see Bininda-Emonds et al., 1999).

DISCUSSION

Although unsupported clades can occur in MRP supertrees (Bininda-Emonds and Bryant, 1998; Pisani and Wilkinson, 2002; Wilkinson et al., 2004), their virtual absence in both the simulated and empirical supertrees (including that of Pisani et al., 2002) is encouraging. Thus far, the only documented examples of unsupported clades occurred predominantly in the consensus setting and with only a limited number of source trees (usually two; see Bininda-Emonds and Bryant,

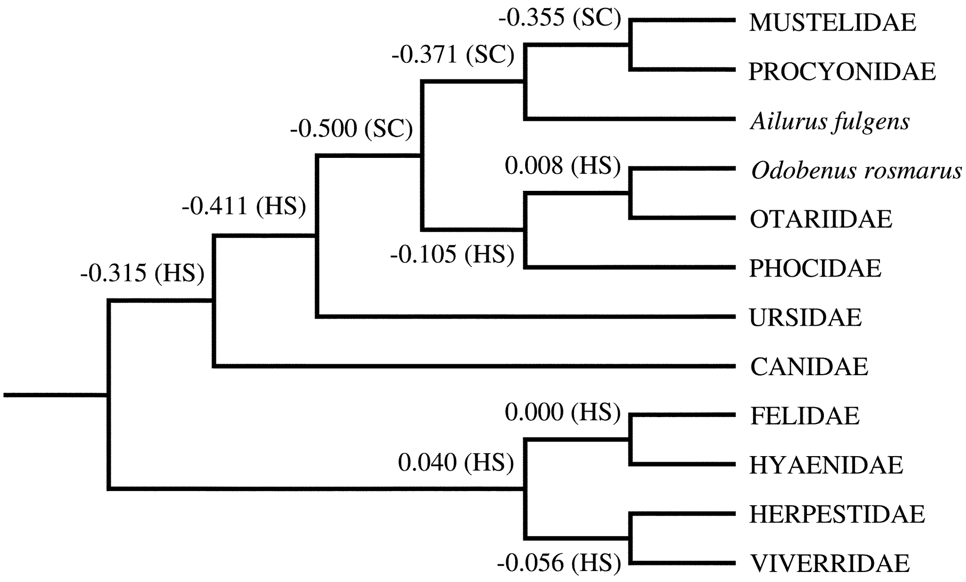

FIGURE 2. Qualitative support and QS_{clade} for the clades in the top-level supertree of the Carnivora (Bininda-Emonds et al., 1999). HS = hard support; SC = soft conflict.

1998; Wilkinson et al., 2001; Goloboff and Pol, 2002). This largely matches the findings of the current simulations. In fact, most examples demonstrating possible limitations in MRP supertree construction (e.g., Purvis, 1995; Wilkinson et al., 2001) have involved a highly restricted number of source trees, which is akin to having a small sample size. However, most published MRP supertrees have used many more source trees than this, and simulation studies have confirmed that MRP performs reasonably under these conditions (Bininda-Emonds and Sanderson, 2001; Bininda-Emonds, 2003; this study).

It is also encouraging that the qualitative support for the clades on a supertree is correlated broadly with the quantitative support inferred for them (e.g., bootstrap frequencies). That is, clades possessing soft or hard conflicts display increasingly reduced measures of support compared with those possessing hard or soft (qualitative) support, such that a researcher would already have decreased confidence in them. Although it is not possible to identify unsupported clades absolutely from such quantitative support measures, such identification can now be done quickly using the protocols in this study. The *QS* index is one of the first support measures that is unaffected by the inherent nonindependence of the matrix representation coding method (see Purvis, 1995). Instead of sampling the individual elements of the matrix, *QS* samples at the more appropriate level of the source trees themselves (see Sanderson, 1995), which are theoretically IID. It is also possible to design a bootstrap resampling scheme that operates at the source tree level (and could also be used to place confidence intervals on the *QS* values). Other nonparametric and parametric supertree bootstrap techniques that resample the primary character data are also under development (Huelsenbeck et al., in prep. Moore et al., in prep.).

The frequency of both hard and soft support among the supertree clades decreases as the source trees overlap to a lesser degree, especially in the absence of any largely complete, but poorly resolved source trees derived from taxonomies (see Bininda-Emonds and Sanderson, 2001). Thus, many supertree clades must be being contradicted by at least one source tree. Given that different phylogenies often disagree with one another, it seems unreasonable in practice to expect a clade in a supertree, especially the larger clades, to not be contradicted by any source tree (as advocated by Goloboff and Pol, 2002). Contradictions also become more likely as the number of source trees is increased. As with strict consensus, a single rogue source tree can obscure widespread agreement among the remaining source trees and dramatically reduce the resolution of the supertree.

For example, except for the smallest supertrees, all supertrees in the composite Carnivora supertree have QS_{tree} values of <0 (Table 4), indicating at least some contradictions between the clades on them and the source trees. More specifically, at the second level of comparison, 148 of the 198 clades (74.7%) in the composite Carnivora supertree are contradicted by at least one source tree (i.e., show a hard mismatch, possibly in addition to a hard match), whereas 189 (95.5%) display either a soft or hard mismatch (data not shown). The percentage of clades with at least one hard mismatch for most of the individual supertrees included in the Carnivora supertree is >50% and usually >80%. This accounting includes the supertree for the Phocidae (87.5%), the topology of which is reasonably uncontroversial. The resulting loss of resolution that would result under the criterion of Goloboff and Pol (2002) belies the general consensus among the source trees as demonstrated by QS_{tree} values that are usually close to zero and by a separate statistical analysis that showed that different categories of source trees (e.g., morphological vs. molecular) were not significantly different from one another (Bininda-Emonds, 2000). The equivalent numbers for the Lagomorpha supertree are slightly higher: 67 clades (86.0%) show at least one hard mismatch, and all 76 clades show at least one soft or hard mismatch among the source trees (data not shown).

This study also confirms the difficulty in inferring the more basal (or more inclusive) relationships in any phylogenetic tree (Bininda-Emonds et al., 2000). With respect to MRP supertrees, clades displaying soft or hard conflicts were progressively larger than those displaying support of any form, which were closer to the tips of the tree. This result stems largely from the increased number of taxa in more inclusive clades and thus the increased number of ways of reconstructing the membership of such clades wrongly (Bininda-Emonds et al., 2000). This result should not be taken to mean that deep relationships (e.g., between phyla) in the Tree of Life cannot be reconstructed accurately. Instead, it means that the more basal clades in any given analysis will be difficult to infer. A possible counterstrategy might be to perform hierarchically nested analyses, as with the Carnivora supertree, thereby limiting the size of the basal clades. However, this approach leads to other problems in the form of assumptions of the monophyly of higher level taxa (see Bininda-Emonds et al., 1998). Another data-directed solution would be to use a method to identify clades that are uncontradicted among all source trees (e.g., semistrict supertrees; Goloboff and Pol, 2002) and use these clades to specify a constraint tree to restrict the search space (A. Purvis, pers.com., 2003). Even so, inferring basal relationships within a tree accurately seems to be a universal problem in phylogenetics that has no simple solution.

ACKNOWLEDGMENTS

The idea for this study came about from numerous e-mail conversations with Davide Pisani. I thank Michael Sanderson for access to computer facilities and Charles Semple, an anonymous reviewer, and especially Peter Lockhart for helpful comments. The Perl scripts used in this study are available at http://www.tierzucht.tum.de/Bininda-Emonds/ or from me on request. Prachi Shah and Davide Pisani of Penn State University have also kindly ported QualiTree.pl as a DOS executable file that can also be downloaded from the above Web site. Support for this research was provided by the German research program BMBF.

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First submitted 26 February 2003; reviews returned 20 June 2003; final acceptance 12 August 2003
Associate Editor: Peter Lockhart