Quartet Cleaning: Improved Algorithms and Simulations

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Abstract

A critical step in all quartet methods for constructing evolutionary trees is the inference of the topology for each set of four species (i.e. *quartet*). It is a well–known fact that all quartet topology inference methods make mistakes that result in the incorrect inference of quartet topology. These mistakes are called *quartet errors*. In this paper, two efficient algorithms for correcting bounded numbers of quartet errors are presented. These "quartet cleaning" algorithms are shown to be optimal in that no algorithm can correct more quartet errors. An extensive simulation study reveals that sets of quartet topologies inferred by three popular methods (Neighbor Joining [15], Ordinal Quartet [14] and Maximum Parsimony [10]) almost always contain quartet errors and that a large portion of these quartet errors are corrected by the quartet cleaning algorithms.

1 Introduction

The explosion in the amount of DNA sequence data now available [3] has made it possible for biologists to address important large scale evolutionary questions [11, 12, 19]. In the analysis of this data an evolutionary tree that describes the evolutionary history of the sequences involved is produced. This tree is typically modeled by an edge–weighted rooted tree where the leaves are labeled by sequences. However, due to the large data sets involved, standard approaches for constructing evolutionary trees such as maximum likelihood [9] and maximum parsimony [18] that exhaustively search the entire tree space are not feasible.

In recent years *quartet methods* for constructing evolutionary trees have received much attention in the computational biology community [1, 2, 4, 8, 14, 17]. Given a quartet of species $\{a, b, c, d\}$ and an evolutionary tree T, the *quartet topology* induced in T by $\{a, b, c, d\}$ is the path structure connecting a, b, c and d in T. Given a quartet $\{a, b, c, d\}$, if the path in T connecting labels a and b is disjoint from the path in T connecting c and d, the quartet is said to be *resolved* and is denoted $ab|cd$. Otherwise, the quartet is said to be *unresolved* and is denoted $(abcd)$. The four possible quartet topologies induced by a quartet are depicted in Figure 1.

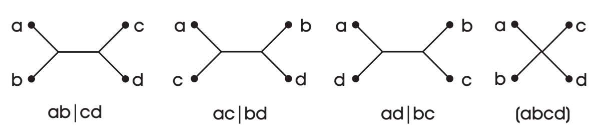

Figure 1: The four quartet topologies for quartet $\{a, b, c, d\}$.

Quartet methods proceed by first estimating the quartet topology induced by each quartet and then recombining these quartet topologies to form an estimate of the true evolutionary tree. This approach is

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based upon the fact that an evolutionary tree T is uniquely characterized by its set Q_T of induced quartet topologies [5] (see Figure 2).

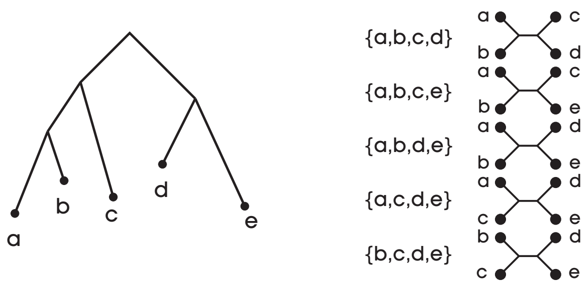

Figure 2: An evolutionary tree T and its set Q_T of induced quartet topologies.

The quartet method paradigm is illustrated in more detail in Figure 3. Let S be a set of sequences that are the product of evolutionary processes. In the example $S = \{a, b, c, d, e, f\}$. Let T be the unknown evolutionary tree that describes the evolutionary history of these sequences. A *quartet topology inference method* is applied to the sequence data to infer the quartet topology for each quartet. This results in a set Q of $\binom{n}{4}$ quartet topologies where $|S| = n$. There are many quartet topology inference methods including maximum parsimony [10], maximum likelihood [9], neighbor joining [15] and the ordinal quartet method [14]. A *quartet recombination method* is applied to Q to obtain an inferred evolutionary tree topology T'. Existing quartet recombination methods are the Q^* method [2], short quartet method [8], and quartet puzzling [17] (see also [1, 13]). Finally, T' is rooted and edge lengths are determined.

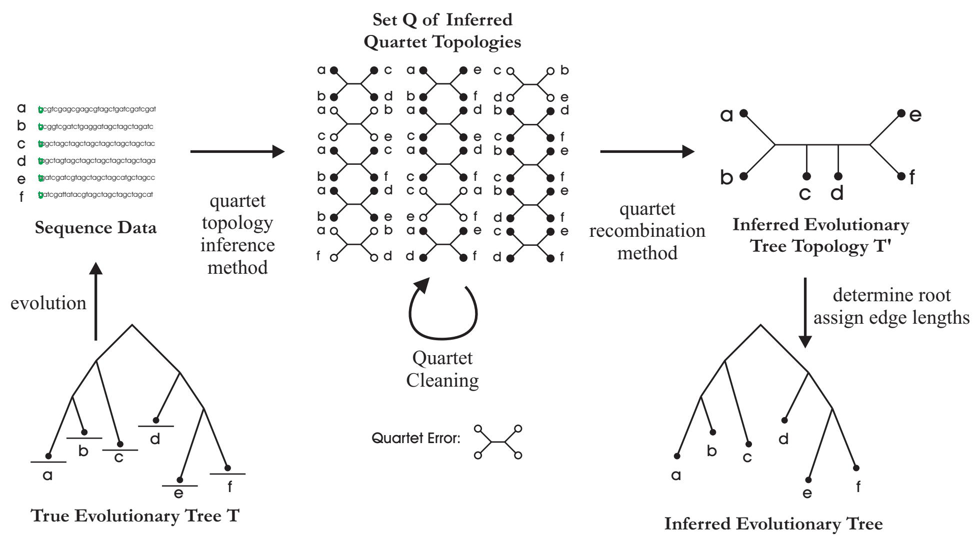

Figure 3: The quartet method paradigm.

The final steps in the quartet method paradigm are well–understood [4, 18]. The computational interest in this paradigm derives from the fact that quartet topology inference methods make mistakes, and so, the set Q of inferred quartet topologies contains *quartet errors*. The quartet $\{a, b, c, d\}$ is a quartet error if $ab|cd \in Q_T$ but $ab|cd \notin Q$. For example, in Figure 3, $\{a, b, c, e\}$ is a quartet error since $ab|ce \in Q_T$ but $ac|be \in Q$. In this sense, Q is an estimate of Q_T. Consequently, the problem of recombining quartet topologies of Q to form an estimate T' of T is typically formulated as an optimization problem:

Maximum Quartet Consistency (MQC)
Instance: Set Q containing a quartet topology for each quartet of labels in S and $k \in \mathcal{N}$.
Question: Is there an evolutionary tree T' labeled by S such that $|Q_{T'} \cap Q| \geq k$?

Problem **MQC** is NP–hard if the input Q is a partial set of quartets, i.e., quartet topologies can be missing [16] (this proof also implies that this version of **MQC** is MAX–SNP hard). Though it was previously known that **MQC** has a polynomial time approximation scheme [13], proving that **MQC** is NP–hard turns out to be more difficult since a complete set of quartets must be constructed. A proof of the following theorem is presented in the appendix:

Theorem 1 MQC *is NP-hard.*

Clearly, the accuracy of the estimate T' depends almost entirely on the accuracy of Q. That is, if Q is not a good estimate of Q_T then the inferred evolutionary tree T' is unlikely to be a good estimate of the true evolutionary tree T. In fact, many quartet recombination methods are very sensitive to quartet errors in Q. For example, the Q^* method [2] and the Short Quartet Method [8] can fail to recover the true evolutionary tree even if there is only one quartet error in Q. Hence, although much effort has been directed towards the development of quartet recombination methods, of prior importance is the development of methods that improve the accuracy of inferred quartet topologies.

There are two approaches for improving the accuracy of inferred quartet topologies. The first is to develop better quartet topology inference methods. This seems unlikely since accurate methods such as maximum likelihood can be employed to infer quartet topology efficiently. The second alternative is to develop methods that detect and correct quartet errors in Q. This process is called "quartet cleaning" (see Figure 3).

This paper presents several results on quartet cleaning:

- Two efficient quartet cleaning algorithms are presented. One of these algorithms is proven to be optimal in its ability to correct quartet errors whereas the other is shown to be robust to the distribution of quartet errors in the true evolutionary tree.
- An extensive simulation is presented that establishes the following:
  - Quartet errors are common, regardless of the quartet topology inference method used. This establishes the *need* for quartet cleaning algorithms.
  - The quartet cleaning algorithms presented are very effective in detecting and correcting quartet errors. In fact, the accuracy of Q can be dramatically improved under most circumstances. This establishes the *applicability* of quartet cleaning algorithms.

These results are described in more detail in Section 1.1 and contrasted with previous results in Section 1.2.

1.1 Terminology and Results

Let S be a set of sequences, Q be a set of quartet topologies and T the true evolutionary tree for S. In this paper it can be assumed that Q contains a quartet topology for each quartet taken from S. Several concepts must be introduced so that quartet cleaning can be discussed in detail.

An edge e in tree T induces the bipartition (A, B) if $T - \{e\}$ consists of two trees where one is labeled by A and the other by B. This is denoted $e = (A, B)$. Let $Q(A, B)$ denote the set of quartet topologies of the form $aa'|bb'$ where $a, a' \in A$ and $b, b' \in B$. An internal vertex v in tree T induces the tripartition (A, B, C) if $T - \{v\}$ consists of three trees labeled by A, B and C, respectively. This is denoted $v = (A, B, C)$. Let $Q(A, B, C)$ denote the union of $Q(A, B)$, $Q(A, C)$ and $Q(B, C)$. Two bipartitions (A, B) and (C, D) are *compatible* if they can be induced in the same tree, *i.e.*, either $A \subseteq C$ or $C \subseteq A$. Similarly, two tripartitions are compatible if they can be induced in the same tree.

In order to assess the performance of quartet cleaning algorithms an understanding of the distribution of quartet errors in T is needed. Let $P_T(a, b)$ be the path between labels a and b in tree T. If $ab|cd$ is induced in T then $P_T(a, c) \cap P_T(b, d)$ is called the *joining path* of $\{a, b, c, d\}$ in T. Define the quartet error $\{a, b, c, d\}$ to be *across* edge e if e is on the joining path of $\{a, b, c, d\}$ in T. Similarly, define the quartet error $\{a, b, c, d\}$

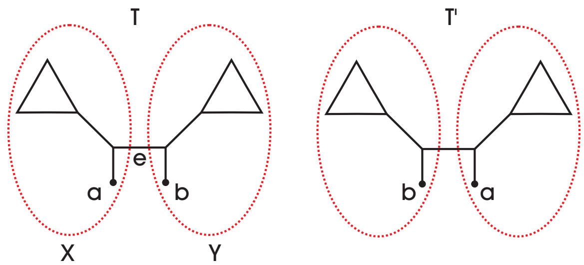

Figure 4: Cleaning bounds.

to be *across* vertex v if v is on the joining path of $\{a, b, c, d\}$ in T. These definitions permit the assignment of quartet errors in Q to edges/vertices of T.

Let $e = (X, Y)$ be the bipartition induced in T as depicted in Figure 4. Observe that Q_T and $Q_{T'}$ differ by quartets of the form $ax|by$ where $x \in X$ and $y \in Y$. It follows that $|Q_T - Q_{T'}| = (|X|-1)(|Y|-1)$. If half of the quartets of the form $\{a, b, x, y\}$ with $x \in X$ and $y \in Y$ have quartet topology $ax|by$ in Q and the other half have quartet topology $bx|ay$ in Q then no quartet cleaning algorithm can guarantee that all quartet errors across e can be corrected under the **MQC** principle of optimality. This implies that $(|X|-1)(|Y|-1)/2$ is an upper bound on the number of quartet errors across an edge of T that can be corrected.

This example motivates the following formulations of quartet edge cleaning:

Local A quartet edge cleaning algorithm has *local cleaning bound* b if it corrects all quartet errors across any edge with fewer than b quartet errors across it.

Global A quartet edge cleaning algorithm has *global cleaning bound* b if it corrects all quartet errors in Q if each edge of T has fewer than b quartet errors across it.

Analogous definitions apply to local and global vertex cleaning algorithms. Local edge/vertex cleaning is more robust that global edge/vertex cleaning since it can be applied to an edge/vertex independently of the number of quartet errors across other edges/vertices. This is a significant feature especially when some edges/vertices have a high number of quartet errors across them. In contrast, global cleaning algorithms are applicable only if all edges/vertices satisfy the cleaning bound.

The example from Figure 4 illustrates that edge/vertex cleaning bounds should not be constant but depend on the bipartition sizes. Hence, an edge $e = (X, Y)$ would have an edge cleaning bound that depends upon $|X|$ and $|Y|$. In particular, the example demonstrates that the optimal edge cleaning bound is $(|X| - 1)(|Y| - 1)/2$.

The first contribution of the paper is an $O(n^4)$ time global edge quartet cleaning algorithm with cleaning bound $(|X|-1)(|Y|-1)/2$. Following the above remarks, this algorithm is optimal in the number of quartet errors it can correct across an edge $e = (X, Y)$. The global edge cleaner is presented in Section 2.1.

The second contribution of the paper is an $O(n^7)$ time local vertex quartet cleaning algorithm with cleaning bound $(|X| - 1)(|Y| - 1)/4$. Although this algorithm has a smaller cleaning bound than the global edge cleaner, it is more robust since it is local. Hence, there are situations where the local vertex cleaner is superior to the global edge cleaner and vice–versa. The local vertex cleaner is presented in Section 2.3.

The third contribution of the paper is an extensive simulation study that assesses the utility of the above quartet cleaning algorithms. This study establishes the following:

- Regardless of the quartet topology inference method used to obtain Q, quartet errors are prevalent in sets of inferred quartet topologies. To establish this three popular quartet topology inference methods are evaluated: maximum parsimony [10], neighbor joining [15] and the ordinal quartet method [14]. This establishes that there is a need for quartet cleaning algorithms.

- The global edge cleaner and the local vertex cleaner are very effective at correcting quartet errors. In particular, the local vertex cleaner is more effective (due to its robustness) and both algorithms dramatically increase the accuracy of the quartet topology set Q.

The simulation study is presented in Section 3.

1.2 Previous Results

The idea of correcting quartet errors in a set of quartet topologies was introduced in [13]. This paper presented a polynomial time global edge quartet cleaning algorithm with cleaning bound $\alpha(|X|-1)(|Y|-1)/2$ where $\alpha > 0$ is a fixed constant. Hence, this algorithm is suboptimal. Although this algorithm has a polynomial time complexity, it is of very high degree, and so, of primarily theoretical interest. The algorithms presented here are more efficient, more effective (the global edge cleaner is optimal) and more robust.

2 Quartet Cleaning Algorithms

2.1 A Global Edge Quartet Cleaning Algorithm

Let S be a set of sequences that evolved on evolutionary tree T and let Q be a set of quartet topologies inferred from S. Assume that there are fewer than $(|A|-1)(|B|-1)/2$ quartet errors across each edge $e = (A, B)$ of T. In other words, assume that

$$|Q(A, B) - Q| < (|A|-1)(|B|-1)/2.$$

The following algorithm constructs T from Q by building T iteratively from the leaves up. Note that for the purposes of this algorithm, a leaf is in R is considered to be a rooted subtree.

Algorithm Global-Clean(Q)
1. Let $R := S$.
2. For every pair of rooted subtrees T_1 and T_2 in R
3. Let A denote the leaf labels of T_1 and T_2.
4. If $|Q(A, S-A) - Q| < (|A|-1)(|S-A|-1)/2$ then
5. Create a new tree T' that contains T_1 and T_2 as its subtrees.
6. Let $R := R - \{T_1, T_2\} \cup \{T'\}$.
7. Repeat step 2 until $|R| = 3$.
8. Connect the three subtrees in R at a new vertex and output the resulting unrooted tree.

Theorem 2 *Algorithm Global-Clean outputs the tree T correctly.*

Proof: See Appendix. ■

Note that if not all edges of T satisfy the global cleaning bound of $(|A|-1)(|B|-1)/2$ then Global–Clean fails to return an evolutionary tree.

A straightforward implementation of Global–Clean results in a time complexity of $O(n^2 \cdot n^4) = O(n^6)$, since each quartet can is checked at most $O(n^2)$ times. Section 2.2 presents a more careful implementation and analysis that yields a linear $O(n^4)$ global edge cleaning algorithm.

2.2 An Efficient Implementation of Global-Clean

The idea is as follows. First, observe that the most time consuming step in Global-Clean is step 4, *i.e.*, the identification of discrepancies between the set of quartet topologies induced by a candidate bipartition and those in the given set Q. Our idea is to avoid considering the same quartet repeatedly as much as possible. In order to achieve this, for each correct bipartition $(A, S-A)$, we record the set $Q(A, S-A) - Q$ using a linked list. Let A_1 and A_2 denote the leaf labels of T_1 and T_2, respectively, considered in step 2 of Global-Clean, and let $A = A_1 \cup A_2$, according to step 3. Then, in step 4, observe that

$$Q(A, S-A) - Q = (Q(A_1, S-A) - Q) \cup (Q(A_2, S-A) - Q) \cup (Q'(A_1, A_2, S-A) - Q),$$

where $Q'(A_1, A_2, S-A)$ denotes the set of all quartet topologies $ab|cd$ with $a \in A_1, b \in A_2$, and $c, d \in S-A$. Since $Q(A_1, S-A)-Q \subseteq Q(A_1, S-A_1)-Q$, we can compute $Q(A_1, S-A)-Q$ from the list for $Q(A_1, S-A_1)$ in at most $|Q(A_1, S-A_1)| = O(n^2)$ time. Similarly, $Q(A_2, S-A)-Q$ can be computed in $O(n^2)$ time. Moreover, at each execution of step 2 (except for the first time), we need only check $O(n)$ new possibilities of joining subtrees: joining the subtree resulting from the previous iteration, say T_1, with each of the $O(n)$ other subtrees, say T_2. Since all the other possibilities have been examined in previous iterations, their outcomes can be easily retrieved. Because step 2 is repeated $n-2$ times, the overall time required for computing the sets $Q(A_1, S-A)-Q$ and $Q(A_2, S-A)-Q$ is $O(n \cdot n \cdot n^2) = O(n^4)$.

Next we show that computing the sets $Q'(A_1, A_2, S-A)-Q$ also globally requires $O(n^4)$ time, thanks to some delicate data structures. For each possible clustering $\{T_1, T_2\}$ of two subtrees, we split the set $Q'(A_1, A_2, S-A)$ into two lists, $Q'_-(A_1, A_2)$ and $Q'_+(A_1, A_2)$, depending on whether a topology contradicts or corresponds to the one in the set Q. That is, $Q'_-(A_1, A_2) = Q'(A_1, A_2, S-A)-Q$. When subtrees T_1 and T_2 are joined, the linked list $Q(A, S-A)$ associated to the new subtree $T' = \{T_1, T_2\}$ is simply obtained by first adding $Q(A_1, S-A)-Q$ and $Q(A_2, S-A)-Q$, which requires $O(n^2)$ time, and then adding the list $Q'_-(A_1, A_2)$, which requires $O(1)$ time if $Q'_-(A_1, A_2)$ (and $Q_+(A_1, A_2)$) is represented as a doubly-linked list. Note that $Q'_-(A_1, A_2)$ need not be scanned as its quartets are distinct from those already added to $Q(A, S-A)-Q$.

The Q'_- and Q'_+ lists of the new possible clusterings involving T' are easy to obtain. If A_i denotes the leaf labels of a subtree $T_i \neq T_1, T_2$, then $Q'_-(A, A_i) = Q'_-(A_1, A_i) \cup Q'_-(A_2, A_i) - Q''(A_1, A_2, A_i)$ and $Q'_+(A, A_i) = Q'_+(A_1, A_i) \cup Q'_+(A_2, A_i) - Q''(A_1, A_2, A_i)$ where $Q''(A_1, A_2, A_i)$ denotes the members of $Q'(A_1, A_2, S-A)$ involving a leaf label in A_i. We proceed by scanning first $Q_-(A_1, A_2)$ and $Q_+(A_1, A_2)$ to remove the occurrences of the elements of $Q''(T_1, T_2, T_i)$ in the Q_- and Q_+ lists of $\{T_1, T_i\}$ and $\{T_2, T_i\}$. Then we merge these disjoint lists in $O(1)$ time to obtain those of $\{T', T_i\}$. Linking together all the occurrences of each quartet in the Q'_- and Q'_+ lists at the beginning of the algorithm enables us to remove each member of $Q''(A_1, A_2, A_i)$ from other Q'_- and Q'_+ lists in $O(1)$ time.

We remark that at any time of the algorithm, each quartet appears only a constant number of times in all Q'_- and Q'_+ lists. If $q = a, b, c, d$ and T_1, T_2, T_3, T_4 are the respective subtrees containing the leaf labels a, b, c, d, then only the 6 clusterings $\{T_1, T_2\}$, $\{T_1, T_3\}$, $\{T_1, T_4\}$, $\{T_2, T_3\}$, $\{T_2, T_4\}$, and $\{T_3, T_4\}$ resolve q, and hence q belongs only to the Q'_- and Q'_+ lists of these 6 clusterings. As each occurrence of a quartet in the Q'_- and Q'_+ lists is checked or removed only once during the algorithm, the maintenance of the Q'_- and Q'_+ lists requires $O(n^4)$ time totally. Now, these lists (and their sizes) enable us to compute $|Q'(A_1, A_2, S-A)-Q| = |Q'_-(A_1, A_2)|$ in $O(1)$ time and thus $|Q(A, S-A)-Q|$ in $O(n^2)$ time, when needed.

As mentioned above, Global-Clean requires $n-2$ clusterings. Before each clustering we have to examine $O(n)$ new candidates, each requiring $O(n^2)$ time. Hence this implementation, summarized in Table 2 (see Appendix), requires $O(n \cdot n \cdot n^2) = O(n^4)$ time.

2.3 A Local Vertex Quartet Cleaning Algorithm

Let S be a set of sequences that evolved on evolutionary tree T and let Q be a set of quartet topologies inferred from S. Define a bipartition (A, B) to be *good* if $|Q(A, B)-Q| \leq (|A|-1)(|B|-1)/4$. A tripartition (A, B, C) is good if each of its three induced bipartitions is good, *i.e.*, $|Q(A, B \cup C)-Q| \leq (|A|-1)(|B \cup C|-1)/4$, $|Q(B, A \cup C)-Q| \leq (|B|-1)(|A \cup C|-1)/4$ and $|Q(C, A \cup B)-Q| \leq (|C|-1)(|A \cup B|-1)/4$.

The following algorithm corrects all quartets errors across vertices of T that induce good tripartitions.

Algorithm Tripartition(Q)

1. Let $Tri(Q) := \emptyset$.
2. For every three labels a, b and c
3. Let $A = \{a\}$; $B = \{b\}$; $C = \{c\}$.
4. For each $w \in S - \{a, b, c\}$.
5. If $aw|bc \in Q$ then $A = A \cup \{w\}$.
6. If $bw|ac \in Q$ then $B = B \cup \{w\}$.
7. If $cw|ab \in Q$ then $C = C \cup \{w\}$.

8.   If (A, B, C) is good then let $Tri(Q) := Tri(Q) \cup \{(A, B, C)\}$.
9. Output $Tri(Q)$.

To show that the above algorithm is a local vertex cleaning algorithm with cleaning bound $(|A|-1)(|B|-1)/4$, it is first proven that $Tri(Q)$ contains all vertex induced tripartitions of T that are good.

Theorem 3 *Let v be a vertex of T that induces tripartition (A, B, C). If (A, B, C) is good then $(A, B, C) \in Tri(Q)$.*

Proof: See Appendix. ∎

Next we show that the tripartitions produced by the Tripartition algorithm are compatible. The following lemma will be useful.

Lemma 1 *Two tripartitions are compatible if and only if their induced bipartitions are all compatible with each other.*

Proof: Omitted. ∎

Theorem 4 *If (A, B, C) and (X, Y, Z) are both in $Tri(Q)$ then they are compatible.*

Proof: See Appendix. ∎

Theorem 3 informs us that $Tri(Q)$ contains all good tripartitions of T and Theorem 4 informs us that $Tri(Q)$ contains only tripartitions compatible with the good tripartitions of T. Let T' be the tree obtained by combining the tripartitions of $Tri(Q)$. Then $Q_{T'}$ is a corrected version of Q. Note that unlike algorithm Global-Clean described in Section 2.1, algorithm Tripartition always produces a tree and this tree is either the same as T or is a contraction of T. A straightforward implementation of the Tripartition algorithm runs in $O(n^3 \cdot n^4) = O(n^7)$ time. An $O(n^5)$ implementation has been found very recently by Della Vedova [7].

3 Simulation Study

In this section, a simulation study is presented that addresses the utility of the quartet cleaning algorithms. In particular, the simulation study addresses questions of need and applicability:

1. How common are quartet errors in sets of inferred quartet topologies?
2. How effective are the quartet cleaning algorithms at correcting quartet errors when they occur?

The approach of the simulation study was to simulate the evolution of sequences on an evolutionary tree T. From these sequences, a set of quartet topologies was inferred using a quartet inference method. This set of quartet topologies was then compared to the actual quartet topologies induced by T. Any quartet whose inferred and actual topologies did not match was considered a quartet error. By mapping these quartet errors back onto the vertices and edges of T, it was possible to establish the number of quartet errors across the vertices and edges of T. From this information it was determined which vertices and edges of T could be cleaned by the global edge and local vertex cleaning algorithms described in this paper.

The simulation study was extensive in that sequences were evolved under a broad range of parameters where the sequence length, evolutionary tree topology and evolutionary rates were varied. As well, three popular quartet inference methods, namely, Neighbor Joining [15], the Ordinal Quartet method [14] and Maximum Parsimony [10] were used to infer the sets of quartet topologies from the simulated sequences.

The details of the simulation study are as follows. For Neighbor Joining and the Ordinal Quartet method, distance matrices were generated from the sequences and corrected relative to the Kimura two–parameter (K2P) model of evolution [18, page 456]. For Maximum Parsimony, the sequences were corrected for transition/transversion bias by weighting the character–state transition matrix appropriately [18, pages 422–423]. Evolutionary trees and sequences were created in a manner similar to [6] using code adapted from program `listtree` in the PAML phylogeny analysis package [20]: Evolutionary tree topologies were generated by adding taxa at random, edge–lengths were assigned according to a specified mean edge–length, and sequences were evolved on these evolutionary trees using the K2P model with transition–transversion bias $\kappa = 0.5$. The simulation examined 1000 randomly–generated topologies on 10 species $\times$ 5 mean edge–lengths per topology $\times$ 100 edge–length sets per topology $\times$ 3 sequence lengths $= 1,500,000$ sequences datasets, where the set of mean edge–lengths considered was $\{0.025, 0.1, 0.25, 0.5, 0.75\}$ and the set of sequence lengths considered was $\{50, 200, 2000\}$. The simulation code consists of a number of C–language and shell–script programs that were compiled and run under the UNIX system on several of the SUN workstations owned by the computational biology group at McMaster University.

The results of the simulation study are summarized in Table 1. Consider how the results address the two questions posed at the beginning of this section.

Firstly, the results clearly establish that quartet errors are prevalent, independent of the quartet topology inference method used. The number of quartet errors decreases as sequence length increases and mean edge–length decreases but remains significant even for sequence length 2000 and mean edge–length 0.025. Hence, there is a definite need for quartet cleaning algorithms.

Secondly, in an absolute sense, the simulation results show that the global edge and local vertex quartet cleaning algorithms can clean significant numbers of trees and vertices per tree, respectively, under moderate mean edge–lengths and large sequence lengths. A comparison of the parenthesized and unparanthesized values in this table establishes the effectiveness of these algorithms. Both algorithms decrease the number of quartet errors significantly under a wide variety of conditions. For example, under the Ordinal Quartet Method with mean edge–length 0.1 and sequence length 200, the increase in accuracy is approximately 25%. When the mean edge–length is large and/or the sequence length is small, the increased accuracy is dramatic.

There are three additional patterns in Table 1 that are worth mentioning:

1. The performance of both quartet cleaning algorithms relative to all three quartet topology inference methods over the mean edge–lengths considered here progresses from a bell–shape (in which both low and high mean edge–lengths reduce performance) to a threshold (in which only high mean edge–lengths reduce performance) as sequence length increases.

2. The performance of both quartet cleaning algorithms under Maximum Parsimony is only better than their performance under Neighbor Joining when sequence–length is high enough and when mean edge–length is neither too small nor too large.

3. The performance of the quartet cleaning algorithms under the Ordinal Quartet method is always better than their performance under Maximum Parsimony and Neighbor Joining. Moreover, the performance of the quartet cleaning algorithms under the Ordinal Quartet method is distinctly better than the performance of the quartet cleaning algorithms under Maximum Parsimony and Neighbor Joining under extreme conditions, *e.g.*, short sequence lengths and/or low or high mean edge–lengths. This confirms the superiority of the Ordinal Quartet method which was also demonstrated by the simulations reported in [14].

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Quartet Inference Method	Mean Edge Length	Global Edge (% of Trees Cleanable) Sequence Length 50	200	2000	Local Vertex (Average % of Vertices per Tree that are Cleanable) Sequence Length 50	200	2000
Maximum Parsimony (Corrected)	0.025	10.48% (0.45%)	62.85% (10.85%)	95.02% (56.67%)	38.98% (29.20%)	76.15% (59.82%)	94.80% (87.27%)
	0.1	38.62% (0.22%)	79.60% (6.17%)	92.34% (27.06%)	55.91% (28.82%)	80.76% (55.57%)	89.90% (74.01%)
	0.25	26.88% (0.01%)	64.44% (0.71%)	78.48% (6.83%)	45.18% (20.09%)	67.96% (37.73%)	77.03% (53.93%)
	0.5	3.25% (0.00%)	23.46% (0.03%)	56.33% (0.87%)	23.50% (10.59%)	43.47% (22.38%)	61.71% (38.42%)
	0.75	0.30% (0.00%)	3.81% (0.00%)	27.46% (0.08%)	13.08% (6.03%)	25.46% (14.11%)	46.57% (28.48%)
Neighbor Joining (Corrected)	0.025	16.36% (0.73%)	70.47% (13.49%)	95.67% (57.18%)	43.79% (32.53%)	79.85% (62.96%)	95.06% (87.46%)
	0.1	47.10% (0.34%)	81.36% (6.92%)	91.72% (24.57%)	60.86% (32.04%)	81.57% (56.65%)	88.97% (72.31%)
	0.25	30.85% (0.02%)	64.18% (0.84%)	76.48% (6.16%)	47.69% (23.19%)	67.50% (38.44%)	75.48% (52.55%)
	0.5	3.91% (0.00%)	22.27% (0.07%)	51.80% (0.79%)	26.91% (16.37%)	43.25% (26.27%)	58.88% (38.08%)
	0.75	0.41% (0.00%)	3.63% (0.00%)	21.47% (0.06%)	16.78% (11.33%)	27.56% (19.41%)	43.48% (30.49%)
Ordinal Quartet Method (Corrected)	0.025	20.49% (1.21%)	74.33% (17.11%)	96.12% (59.25%)	47.79% (37.36%)	82.19% (66.38%)	95.45% (88.19%)
	0.1	64.15% (1.21%)	86.91% (12.38%)	93.84% (34.53%)	71.92% (40.32%)	86.32% (63.45%)	91.50% (78.00%)
	0.25	63.64% (0.16%)	81.72% (2.42%)	87.31% (13.82%)	68.85% (30.29%)	81.13% (47.25%)	85.44% (65.10%)
	0.5	39.03% (0.04%)	67.26% (0.19%)	81.75% (1.82%)	52.41% (23.67%)	69.18% (32.06%)	79.47% (45.22%)
	0.75	12.32% (0.00%)	35.18% (0.01%)	63.60% (0.16%)	34.28% (18.56%)	49.52% (24.86%)	66.47% (33.28%)

Table 1: Performance of the Quartet Cleaning Algorithms Under Simulation. The unparanthesized number is the average percent of all evolutionary trees (vertices per evolutionary tree) that have no quartet errors (no quartet errors across them) after global edge cleaning (local vertex cleaning) has been applied. The parenthesized number is the average percent of all evolutionary trees (vertices per evolutionary tree) that have no quartet errors (no quartet errors across them) before quartet cleaning is applied.

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Appendix

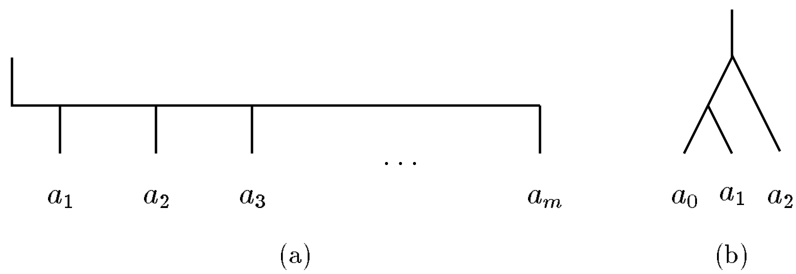


Figure 5: Constructions employed in the proof of Theorem 1.

Proof of Theorem 1:

We show that **MQC** is NP-hard by a reduction from the following problem:

Quartet Compatibility

INSTANCE: A (possibly incomplete) set Q of quartets on label set L.

QUESTION: Is Q compatible, i.e. does there exist a tree T such that $Q \subseteq Q_T$?

This problem is known to be NP-hard [16]. Given an instance of **Quartet Compatibility** with the quartet set Q defined on the label set L, $n = |L|$, we will construct an instance of **MQC** with the complete quartet set Q' defined on the label-set L' such that there is a tree T realizing Q with no error if and only if there is a tree T' realizing Q' with at most $g(n)m + f(n)$ errors. We will specify $f(n)$, $g(n)$ and m later.

The main idea behind this proof is to add quartets that are not specified in Q to create Q' such that no matter what the shape of the tree T', precisely one third of these added quartets are correct. In order to do this, we add the large linear tree M on m leaves (see Figure 5(a)) to the optimal tree T, where m is a number that is both divisible by three and larger than 81 times the number of unspecified quartet topologies in Q, e.g., $>> n^8$. The purpose of M is to enforce certain useful structures in T'.

We construct L' and Q' as follows: Add the m leaf-labels in M to L', and for each $a \in L$, create three new labels a_0, a_1, a_2 and add them to L' (note that we do not add the original labels in L to L'). We want to specify Q' such that (1) each such created triplet of labels occur together in a subtree of T' as in Figure 5(b) and (2) if T is the optimal tree realizing Q then T' is a tree such that M is attached at an arbitrary point to T and each label a in T is replaced by a subtree with leaves a_0, a_1, a_2 as in Figure 5(b). Intuitively, Q' must be constructed to enforce the following conditions:

- M appears intact in T' as in Figure 5(a).
- Each created triplet of labels a_0, a_1, a_2 occur together in a subtree of T' as in Figure 5(b), with nothing else inserted between them.
- The quartet topologies in Q extend naturally to Q'. That is, if $ab|cd \in Q$, then add $a_i b_j|c_k d_l$ to Q' for all $0 \leq i, j, k, l \leq 2$.
- For all quartets whose relationships are not specified in Q or implied by M or the three way splitting, we add quartet topologies in Q' such that precisely one third of these new quartet topologies are satisfied in T', regardless of the structure of T'. This is the difficult part.

Such a Q' is constructed as follows. For every four distinct labels in L':

1. If they are all in M, specify the quartet topology according to the structure of M in Figure 5(a).
2. If exactly three of them are in M, specify the quartet topology as $ax|yz$ for $x, y, z \in M$ and $a \notin M$.
3. If exactly two of them are in M, specify the quartet topology as $ab|xy$ for $x, y \in M$ and $a, b \notin M$.

4. If exactly one of them is in M, we consider two subcases:

   (a) If among the three labels that are not in M, either all three or some two of them are for the same label a in L, then the quartet topology can be specified according to the required structure of T' described above.

   (b) If the three labels a, b, c that are not in M are all for distinct labels in L, then partition M into subsets M_1, M_2, and M_3 such that $|M_1| = |M_2| = |M_3| = \frac{1}{3}m$ and add the sets of quartet topologies $\{x_1a|bc \mid x_1 \in M_1\}$, $\{x_2b|ac \mid x_2 \in M_2\}$, and $\{x_3c|ab \mid x_3 \in M_3\}$ to Q'.

5. Finally, if none of them is in M, we consider three subcases:

   (a) If at least two of them are for the same label in L, then this quartet topology can be specified as before.

   (b) If they are all from different labels a, b, c, d in L and there is a resolved quartet in Q for a, b, c, d, then specify the same partition in Q'. That is, if $ab|cd$ in Q, then add the set of quartet topologies $\{a_ib_j|c_kd_l \mid 0 \le i, j, k, l \le 2\}$ to Q'.

   (c) If they are all from different labels a, b, c, d in L and there is no resolved quartet in Q for a, b, c, d, we take care of such quartets collectively. For each set of original labels a, b, c, d, we have a_i, b_i, c_i, d_i with $i = 0, 1, 2$. These form $3^4 = 81$ different quartets (one from each type). We divide them into $81/3 = 27$ disjoint tracks. Each such track i, j, k, l contains three quartets:

$$
\begin{gathered}
a_i, b_j, c_k, d_l \\
a_{i+1 \bmod 3}, b_{j+1 \bmod 3}, c_{k+1 \bmod 3}, d_{l+1 \bmod 3} \\
a_{i+2 \bmod 3}, b_{j+2 \bmod 3}, c_{k+2 \bmod 3}, d_{l+2 \bmod 3}
\end{gathered}
$$

   For each track, that track's quartet topologies are specified as follows:

$$
\begin{gathered}
a_i b_j | c_k d_l \\
a_{i+1 \bmod 3} c_{k+1 \bmod 3} | b_{j+1 \bmod 3} d_{l+1 \bmod 3} \\
a_{i+2 \bmod 3} d_{l+2 \bmod 3} | b_{j+2 \bmod 3} c_{k+2 \bmod 3}
\end{gathered}
$$

Obviously, the quartet topologies specified in items 1, 2, 3, 4(a), 5(a) and 5(b) are not quartet errors in T' if M stays as in Figure 5(a) and for each $a \in L$, its associated triplet of labels $a_0, a_1, a_2 \in L'$ occur together in a subtree of T' as in Figure 5(b).

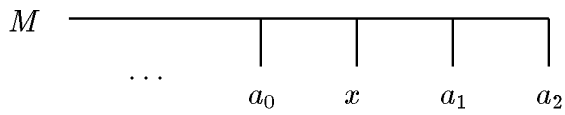
Claim 1 *If M does not split, item 4(b) introduces precisely $g(n)m$ quartet errors, where $g(n) = 18\binom{n}{3}$.*

Proof: There are n labels in L, each splitting into 3 labels in L'. We thus have n sets, each containing three elements. The different number of ways of choosing three sets from n sets is $\binom{n}{3}$, and given three sets, there are 27 ways to form different triples by choosing one label from each set. For each such triple, there are m ways of forming a quartet along the lines sketched in item 4(b), and precisely $m/3$ of these quartets will be correct relative to T'. Hence, item 4(b) introduces precisely $\binom{n}{3} \times 27 \times \frac{2m}{3} = 18\binom{n}{3}m = g(n)m$ quartet errors. ■

Claim 2 *Let $q(n)$ be the number of quartets on L that are not specified. If M does not split, then for each $a \in L$, its associated triplet of labels $a_0, a_1, a_2 \in L'$ occur together in a subtree of T' as in Figure 5(b) and item 5(c) introduces precisely $f(n) = 54q(n)$ quartet errors.*

Proof: Suppose for some $a \in L$, $a_0, a_1, a_2 \in L'$ do not occur together in a subtree as in Figure 5(b). Assume without loss of generality that there is a single element x separating these three elements in T' as shown below:

Under these circumstances, the quartet y, a_0, x, a_1 for any $y \in M$ corresponds to a quartet error, and as there are at least m such y, there are at least m quartet errors. Such errors can only occur among the quartets



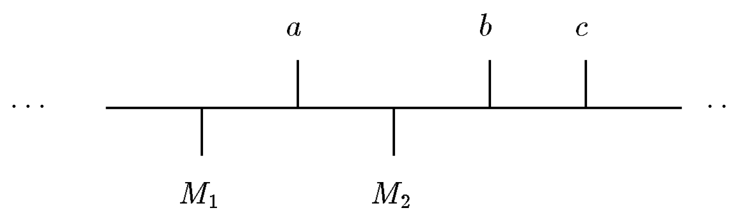
in Q' that correspond to quartets that were not specified in Q. However, as there are $q(n)$ such quartets in Q, each of which corresponds to 81 quartets in Q', and m is larger than $81q(n)$ by definition, this is a contradiction. Hence, no such structure can exist in T'.

Given that each triplet of labels corresponding to an label in L occur together in a subtree in T' as in Figure 5(b), precisely one third of the quartets specified in item 5(c) for each unspecified quartet in Q are correct. As there are $81 \times \frac{2}{3} = 54$ quartet errors associated with each such unspecified quartet in Q, there are $54q(n)$ such errors in total. Hence, item 5(c) introduces precisely $54q(n) = f(n)$ quartet errors. ■

Finally, we have to show that M stays unchanged as in Figure 5(a) in the optimal tree T' for Q'.

Lemma 2 *In the optimal tree T' realizing Q', M stays intact as in Figure 5(a).*

Proof: Splitting M might only reduce the error term in Claim 1. In order to reduce such errors in Claim 1, it must be the case that the following picture is true.



Otherwise, if a is on the right side of M_2 and together with b, c, then still only one third of them are correct, it only creates more errors within M. Note: in this picture, we conceptually collected everything not in M_1 as M_2. I.e. M_2 may be several small trees and also may appear anywhere. Furthermore, a can be on either side of M_1.

Thus, for each x we allot to M_2, we save at most $g(n)$ errors for Item 4, but this creates $\Omega(m)$ more errors because all the quartets like

$$m_1 a | m_2 b$$

are erroneous quartets for $m_1 \in M_1$ and $m_2 \in M_2$. And since m is extremely large by definition, this is not worth it. Hence the optimal way is to have M stay in one piece and in the shape of Figure 5(a). ■

From the above, we conclude that Q is compatible if and only if the optimal tree T' realizing Q' violates at most $g(n)m + f(n)$ quartets in Q'. ■

Proof of Theorem 2:

To see that the algorithm works first observe that if T_1 and T_2 are in R and they should be joined then the algorithm does so because of the error bound imposed on all edges of T.

Conversely, suppose that the algorithm makes a mistake in joining T_1 and T_2. Let A denote the leaf labels of T_1 and let B denote the leaf labels of T_2. Hence, there is an edge e in T such that e induces the bipartition $(A_e, B_e) = (A \cup A', B \cup B')$ for some nonempty sets A', B', as illustrated in Figure 6.

Since the edge e satisfies the error bound,

$$|Q(A \cup A', B \cup B') - Q| < (|A \cup A'| - 1)(|B \cup B'| - 1)/2.$$

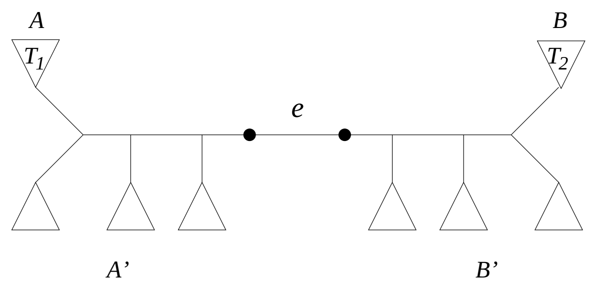


Figure 6: Algorithm Global-Clean joins T_1 and T_2 incorrectly.

Since the algorithm joins T_1 and T_2,

$$|Q(A \cup B, A' \cup B') - Q| < (|A \cup B| - 1)(|A' \cup B'| - 1)/2.$$

Note that the differences between set $Q_1 = Q(A \cup A', B \cup B')$ and set $Q_2 = Q(A \cup B, A' \cup B')$ are the quartets of the form $A \times B \times A' \times B'$. Furthermore, if q is a quartet inducing a topology in Q_1 but not in Q_2 (or vice-versa) then q induces a topology in $Q_1 - Q$ or $Q_2 - Q$. It follows that

$$|Q_1 \oplus Q_2| \leq |Q_1 - Q| + |Q_2 - Q|.$$

Hence we obtain

$$|A| \cdot |A'| \cdot |B| \cdot |B'| < (|A| + |A'| - 1)(|B| + |B'| - 1)/2 + (|A| + |B| - 1)(|A'| + |B'| - 1)/2$$

It is easy to verify that the above inequality does not hold for any $|A|, |B|, |A'|, |B'| \geq 1$. Therefore, we conclude that the algorithm cannot join subtrees incorrectly. ∎

Proof of Theorem 3:

Proof: If $(A, B, C) \notin Tri(Q)$ then for all triples $a \in A$, $b \in B$ and $c \in C$, there exists a $w \in S - \{a, b, c\}$ such that $\{a, b, c, w\}$ is a quartet error in Q. In this case we say that the quartet $\{a, b, c, w\}$ *ruins* the triple (a, b, c). Note that $\{a, b, c, w\}$ also ruins exactly one of the triples (a, b, w), (a, w, c) and (w, b, c) depending on whether $w \in C$, $w \in B$ or $w \in A$. That is, a quartet error $\{a, b, c, w\}$ ruins exactly two triples from $A \times B \times C$.

It follows that there must be $|A| \cdot |B| \cdot |C|/2$ quartet errors across v. Since (A, B, C) is good, the number of quartet errors across v is bounded above by

$$((|A| - 1)(|B \cup C| - 1) + (|B| - 1)|A \cup C| - 1) + (|C| - 1)(|A \cup B| - 1))/4.$$

This implies that

$$\begin{aligned} |A| \cdot |B| \cdot |C|/2 \quad < \quad & ((|A| - 1)(|B \cup C| - 1) + (|B| - 1)|A \cup C| - 1) + \\ & (|C| - 1)(|A \cup B| - 1)) \ / \ 4, \end{aligned}$$

which is not true for any $|A|, |B|, |C| \geq 1$. ∎

Proof of Theorem 4:

Proof: Let (A, B) and (X, Y) be two of the bipartitions induced by the tripartitions in $Tri(Q)$. By the above lemma, it suffices to show that (A, B) and (X, Y) are compatible.

Suppose that (A, B) and (X, Y) are incompatible. Then there must exist nonempty subsets A_1, A_2, B_1, B_2 such that $A = A_1 \cup A_2$, $B = B_1 \cup B_2$, $X = A_1 \cup B_1$, and $Y = A_2 \cup B_2$ Let $a_i = |A_i|$ and $b_i = |B_i|$ for $i = 1, 2$. Since (A, B) and (X, Y) are both good, then

$$|Q(A, B) - Q| < (|A| - 1)(|B| - 1)/4 = (a_1 + a_2 - 1)(b_1 + b_2 - 1)/4$$

{ *Initialization* }
1. $R := S$.
2. For each rooted subtree T_1 in R
3. $\quad Q(A_1, S - A_1) := \emptyset$.
3. For each pair of rooted subtrees T_1 and T_2 in R
4. $\quad Q_-(A_1, A_2) := \emptyset$; $Q_+(A_1, A_2) := \emptyset$.
5. For each $q = ab|cd \in Q$
6. $\quad$ Let T_1, T_2, T_3, T_4 be the subtrees in R containing respectively a, b, c, d.
7. $\quad Q_+(A_1, A_2) \xleftarrow{+} \{q\}$; $Q_+(A_3, A_4) \xleftarrow{+} \{q\}$
8. $\quad Q_-(A_1, A_3) \xleftarrow{+} \{q\}$; $Q_-(A_1, A_4) \xleftarrow{+} \{q\}$; $Q_-(A_2, A_3) \xleftarrow{+} \{q\}$; $Q_-(A_2, A_4) \xleftarrow{+} \{q\}$.

{ *Subtree clustering phases* }
9. While $\exists \{T_1, T_2\}$ in R s.t. $|Q(A, S-A) - Q| < (|A|-1)(|S-A|-1)/2$, where $A = A_1 \cup A_2$
10. $\quad$ Create a new subtree T' that contains T_1 and T_2 as its subtrees.
11. $\quad R := R - \{T_1, T_2\} \cup \{T'\}$
12. $\quad Q(A, S-A) - Q \;\longleftarrow\; Q(A_1, S-A_1) - Q \;-\; \{q = a, a', c, d \text{ s.t. } a, a' \in A_1, c \in A_2\}$
13. $\quad Q(A, S-A) - Q \;\xleftarrow{+}\; Q(A_2, S-A_2) - Q \;-\; \{q = b, b', c, d \text{ s.t. } a, a' \in A_2, c \in A_1\}$
14. $\quad Q(A, S-A) - Q \;\xleftarrow{+}\; Q'_-(A_1, A_2)$.
15. $\quad$ For each $q = a, b, c, d \in Q'(A_1, A_2, S-A)$ $(\equiv Q'_-(A_1, A_2) \cup Q'_+(A_1, A_2))$
16. $\quad\quad$ Let T_1, T_2, T_3, T_4 be the subtrees in R containing respectively a, b, c, d.
17. $\quad\quad$ If $T_3 \neq T_4$ then
18. $\quad\quad\quad$ remove q from the Q'_- and Q'_+ lists of the joins $\{A_1, A_3\}, \{A_1, A_4\}, \{A_2, A_3\}, \{A_2, A_4\}$.
19. $\quad$ For each rooted subtree $T_i \neq T_1, T_2$ in R
20. $\quad\quad Q'_-(A, A_i) := Q'_-(A_1, A_i) \cup Q'_-(A_2, A_i)$
21. $\quad\quad Q'_+(A, A_i) := Q'_+(A_1, A_i) \cup Q'_+(A_2, A_i)$.

Table 2: An efficient implementation of Global-Clean.

and

$$|Q(X, Y) - Q| < (|X| - 1)(|Y| - 1)/4 = (a_1 + b_1 - 1)(a_2 + b_2 - 1)/4.$$

On the other hand, we have

$$|Q(A, B) - Q| + |Q(X, Y) - Q| \geq |Q(A, B) \oplus Q(X, Y)| = a_1 a_2 b_1 b_2.$$

Taken together, this implies that

$$a_1 a_2 b_1 b_2 < (a_1 + a_2 - 1)(b_1 + b_2 - 1)/4 + (a_1 + b_1 - 1)(a_2 + b_2 - 1)/4.$$

However, this inequality does not hold for any $a_1, a_2, b_1, b_2 \geq 1$. ■