THE NEIGHBOR-NET ALGORITHM

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Abstract. The neighbor-joining algorithm is a popular phylogenetics method for constructing trees from dissimilarity maps. The neighbor-net algorithm is an extension of the neighbor-joining algorithm and is used for constructing split networks. We begin by describing the output of neighbor-net in terms of the tessellation of $\overline{\mathcal{M}}_0^n(\mathbb{R})$ by associahedra. This highlights the fact that neighbor-net outputs a tree in addition to a circular ordering and we explain when the neighbor-net tree is the neighbor-joining tree. A key observation is that the tree constructed in existing implementations of neighbor-net is not a neighbor-joining tree. Next, we show that neighbor-net is a greedy algorithm for finding circular split systems of minimal balanced length. This leads to an interpretation of neighbor-net as a greedy algorithm for the traveling salesman problem. The algorithm is optimal for Kalmanson matrices, from which it follows that neighbor-net is consistent and has optimal radius $\frac{1}{2}$. We also provide a statistical interpretation for the balanced length for a circular split system as the length based on weighted least squares estimates of the splits. We conclude with applications of these results and demonstrate the implications of our theorems for a recently published comparison of Papuan and Austronesian languages.

1. Introduction

The neighbor-net algorithm was introduced by Bryant and Moulton in [10]. It is a method for constructing split networks [24] from distance measurements, and has been used for evolutionary analyses in linguistics [8, 26] and phylogenetics [32]. Neighbor-net is gaining in popularity because it is as fast as distance based methods for tree construction, and the split networks output by the algorithm are informative for studying conflicting signals in data. The interpretations of split networks are based on T-theory [3, 25], which is an active research area within mathematics.

Despite the intuitive appeal of split networks for data analysis, a criticism of their use in phylogenetics, and of the neighbor-net algorithm in particular, has been the lack of an obvious tree interpretation. Moreover, although it was remarked in [10] that "neighbor-net is based on the neighbor-joining algorithm of Saitou and Nei [43]", this was meant to indicate analogy at a high level: neighbor-net and neighbor-joining are both agglomerative algorithms, they have similar selection criteria, and they are both consistent. However despite the obvious similarities between neighbor-net and neighbor-joining, there has been no direct link established between the outputs of the algorithms.

Key words and phrases. Neighbor-net, neighbor-joining, circular decomposable metric, traveling salesman problem, Kalmanson conditions, balanced length, minimum evolution, splits network.

It is desirable to establish a mathematically precise connection because there have been a number of recent papers "explaining" neighbor-joining [31], both in terms of showing what it optimizes [21] and why it works well in practice [38]. The lack of informative theorems about neighbor-net coupled with the difficulties in mastering T-theory have contributed to a sense that interpretations of neighbor-net results "remain messy and subject to a certain degree of subjectivity[1]."

We describe the precise connection between neighbor-net and neighbor-joining in Section 2, and in Section 5 we show that our observation can be used to allay concerns that neighbor-net provides no direct phylogenetic *tree* information. Our result also provides an interpretation of $\overline{\mathcal{M}}_0^n(\mathbb{R})$ as the space of phylogenetic networks. In Section 3 we show that neighbor-net is a greedy algorithm for the traveling salesman problem that minimizes the balanced length of the split system at every step. This extends the notion of balanced length in [45] and the results of [21] where it was shown that neighbor-joining greedily optimizes the balanced length of a tree. In Section 4, we prove that neighbor-net is optimal for Kalmanson dissimilarity maps. This establishes new proofs for results of [14, 15, 17], and provides an analog of Atteson's neighbor-joining robustness theorem [2] for neighbor-net.

2. The mathematics

The main objects of study in this paper are a class of discrete metric spaces called *circular decomposable metrics* that include tree metrics as a special case. We begin with an introduction to some fundamental results about these metric spaces. Their study is part of T-theory, and we refer the reader to [25] for a more thorough introduction and survey of the subject. Throughout the paper, $X = \{1, \dots, n\}$ denotes the finite set on which metrics are defined.

Definition 1. A *split* $S = \{A, B\}$ is a partition of X into two non-empty blocks. A set of splits is called a *split system*. The split metric determined by S is the pseudo-metric

$$\delta_S = \begin{cases} 0 & \text{if } \{x, y\} \subseteq A \text{ or } \{x, y\} \subseteq B. \\ 1 & \text{otherwise.} \end{cases}$$

Definition 2. A split system $\mathcal{S}$ is *pairwise compatible* if for every pair of distinct splits $S_1 = \{A, B\}$, $S_2 = \{A', B'\}$ in $\mathcal{S}$, at least one of the intersections

$$A \cap A',\ A \cap B',\ B \cap A',\ B \cap B'$$

is empty.

Definition 3. A *dissimilarity map* on $X = \{1, \dots, n\}$ is a function $\delta : X \times X \to \mathbb{R}$ that satisfies $\delta(i, j) = \delta(j, i) \geq 0$ and $\delta(i, i) = 0$. A dissimilarity map δ satisfies the *four point*

[1]The statement appears in the specific context of a commentary on a paper describing the classification of Bantu languages [37]; we believe that it reflects prevailing sentiment about the neighbor-net algorithm and its utility for evolutionary analyses.

condition if for every four elements $i, j, k, l \in X$, two of the three terms in the following list are equal and greater than the third:

$$\delta(i,j) + \delta(k,l),\ \delta(i,k) + \delta(j,l),\ \delta(i,l) + \delta(j,k).$$

Theorem 4 ([44])**.** *The following are equivalent statements about $\delta : X \times X \to \mathbb{R}$:*

(1) *There exists a split system $\mathcal{S}$ such that every pair of distinct splits in $\mathcal{S}$ is pairwise compatible, and $\delta = \sum_{S \in \mathcal{S}} \lambda_S \delta_S$ where $\lambda_S \geq 0$ for all $S \in \mathcal{S}$.*
(2) *δ is a metric and satisfies the four point condition.*

There is a canonical median graph associated with a split system called the *Buneman graph* [11]. The Buneman graph of a pairwise compatible split system is a tree, and therefore, in light of Theorem 4, metrics satisfying the four point condition are called *tree metrics*. They are precisely the metrics $\delta : X \times X \to \mathbb{R}$ for which there is an edge weighted tree whose leaves are labeled by X, and for which $\delta(i,j)$ is the "additive distance" between i and j in the tree.

Theorem 4 provides the necessary ingredients for describing the input and output of the neighbor-joining algorithm. Specifically, neighbor-joining is an efficient algorithm for evaluating a certain function from the set of dissimilarity maps to pairwise compatible split systems. A key feature of the algorithm, is that the steps explicitly construct the Buneman tree associated with the output.

The neighbor-net algorithm is similarly explained in terms of certain split systems and metrics. The key concept is that of a circular ordering for a finite set X.

Definition 5. A *circular ordering* $\pi = \{x_1, \ldots, x_n\}$ is a bijection between X and the vertices of the n-cycle C_n such that x_i and x_{i+1} are adjacent vertices of C_n. We adopt the convention that $x_{n+1} = x_1$.

Given a circular ordering π, let $W_\pi = \{\{\{x_i, x_j\}, \{x_k, x_l\}\} : i < j < k < l \text{ or } l < i < j < k\}$. Note that W_π is a set consisting of pairs of sets constructed from quartets. In what follows we use the notation $(ij;kl)$ to denote the quartet $\{\{x_i, x_j\}, \{x_k, x_l\}\}$.

Definition 6. A split system $\mathcal{S}$ is *circular* with respect to a circular ordering $\pi = \{x_1, \ldots, x_n\}$ if every split $S \in \mathcal{S}$ is of the form

$$S = \{\{x_{i+1}, \ldots, x_j\}, \{x_{j+1}, \ldots, x_i\}\} \text{ for some } i < j.$$

Note that every pairwise compatible split system is circular.

Definition 7. A dissimilarity map δ satisfies the *Kalmanson conditions* [35] with respect to a circular ordering π if for every $i < j < k < l$,

$$\begin{aligned}\delta(x_i, x_j) + \delta(x_k, x_l) &\leq \delta(x_i, x_k) + \delta(x_j, x_l),\\ \delta(x_i, x_l) + \delta(x_j, x_k) &\leq \delta(x_i, x_k) + \delta(x_j, x_l).\end{aligned}$$

Given a dissimilarity map δ that satisfies the Kalmanson conditions with respect to a circular ordering π, we let $W_\delta = \{(ij;kl) : \delta(x_i, x_j) + \delta(x_k, x_l) < \delta(x_i, x_k) + \delta(x_j, x_l) \text{ for } i < j < k < l \text{ or } l < i < j < k\}$. Note that $W_\delta \subseteq W_\pi$ is a set of quartets given by the strict Kalmanson inequalities.

Theorem 8 ([13, 15]). *The following are equivalent statements about* $\delta : X \times X \to \mathbb{R}$*:*

1. *There exists a circular ordering* π *and a split system* $\mathcal{S}$ *so that* $\delta = \sum_{S \in \mathcal{S}} \lambda_S \delta_S$ *where every split* $S \in \mathcal{S}$ *is circular with respect to* π *and* $\lambda_S \geq 0$ *for all* $S \in \mathcal{S}$.
2. δ *is a metric and satisfies the Kalmanson conditions with respect to* π.

Moreover, a quartet $(ij;kl) \in W_\delta$ *iff there exists a split* S *with* $\lambda_S > 0$ *such that* i, j *and* k, l *are in different blocks of* S.

Metrics satisfying condition (1) of Theorem 8 are called *circular decomposable metrics*, and it is possible to represent them using *split graphs*. These are described in detail in [10]. Here we merely illustrate the idea with an example (Figure 1(a,b)). Each class of parallel edges corresponds to one split $S \in \mathcal{S}$ and the length of the edges in a class are given by the λ_S. Split graphs are not necessarily unique, but they provide a useful way to visualize a circular decomposable metric. The neighbor-net algorithm outputs a circular ordering for the purpose of visualizing a circular decomposable metric associated to it using split graphs. The algorithm is *agglomerative*, which means that the circular ordering is constructed iteratively. The boxed Algorithm 1 describes the details of the algorithm. The terms used in its description are defined below:

Definition 9. Let G be a subgraph of the cycle C_n with n vertices and m components. The graph G is called the *circular ordering graph*. A *partial circular ordering* $\mathcal{C}$ consists of the graph G together with a bijection between X and the vertices of G.

Equivalently, a partial circular ordering is a partition $\mathcal{C}$ of X into ordered sets $\mathcal{C} = \{C_1, \ldots, C_m\}$ where each $C_r \subseteq X$ and i, j are adjacent elements in C_r for some r iff i, j correspond to adjacent vertices in G. We use the notation $\hat{C}_r$ to denote the vertices of degree 0 or 1 in the subgraph corresponding to C_r.

Definition 10. Let $\mathcal{C}$ be a partial circular ordering with $|\mathcal{C}| = m$. A *weighting* for $\mathcal{C}$ consists of a function $\mu : X \to \mathbb{R}$ such that $\mu(i) \geq 0$ for all $i \in X$, and for each $r \in \{1, \ldots, m\}$, $\sum_{i \in C_r}, \mu(i) = 1$ and $\mu(i) > 0$ for all $i \in \hat{C}_r$. We define

$$\delta(C_r, C_s) := \sum_{i \in C_r, j \in C_s} \mu(i)\mu(j)\delta(i,j), \text{and} \quad (1)$$

$$\delta(x, C_r) := \sum_{i \in C_r} \mu(i)\delta(x,i) \quad (2)$$

Note that if $|\mathcal{C}| = |X|$ then there is only one weighting for $\mathcal{C}$, i.e., $\mu(i) = 1$ for all i. Next, we introduce two types of weightings that lead to interesting neighbor-net algorithms in Sections 3 and 5.

Definition 11. A weighting $\mu : X \to \mathbb{R}$ is a *TSP weighting* if, for all $i \in X$, $\mu(i) = 0$ for all $i \notin \hat{C}_r$.

These weightings lead to aggressive greedy algorithms for the traveling salesman problem (Theorem 23).

Algorithm 1: Neighbor-net algorithm

Data : A dissimilarity map $\delta : X \times X \to \mathbb{R}$.
Result: Circular ordering $\pi : X \to C_n$ together with a split system $\mathcal{T}$ of $n-1$ pairwise compatible splits that are circular with respect to π.
Let G be the disjoint union of n vertices and $\mathcal{C}$ the partial circular ordering with graph G. Let $\mu : X \to \mathbb{R}$ be the weighting for $\mathcal{C}$.
while $|\mathcal{C}| > 1$ **do**
  for $i, j \in \binom{|\mathcal{C}|}{2}$ **do**
    Set
    $Q_\delta(C_r, C_s) = (|\mathcal{C}| - 2)\delta(C_r, C_s) - \sum_{t \in \mathcal{C} \setminus \{C_r\}} \delta(C_r, C_t) - \sum_{C_t \in \mathcal{C} \setminus \{C_s\}} \delta(C_t, C_s)$.
  end
  [**Selection step part 1**] Choose a pair $C_{r^*}, C_{s^*} \in \mathcal{C}$ that minimizes Q_δ;
  for $i \in \hat{C}_{r^*},\ j \in \hat{C}_{s^*}$ **do**
    Set $\hat{Q}_\delta(i,j) = (|\mathcal{C}| - 4 + |\hat{C_{r^*}}| + |\hat{C_{s^*}}|)\delta(i,j) - \sum_{t \neq r^*, s^*} \delta(i, C_t) - \sum_{t \neq r^*, s^*} \delta(j, C_t) - \sum_{k \in (C_{r^*} \cup C_{s^*}) \setminus \{i\}} \delta(i,k) - \sum_{k \in (C_{r^*} \cup C_{s^*}) \setminus \{j\}} \delta(j,k)$.
  end
  [**Selection step part 2**] Choose the pair $i^* \in \hat{C}_{r^*},\ j^* \in \hat{C}_{s^*}$ that minimizes $\hat{Q}_\delta$;
  [**Merge step**] Let u, v be the vertices in the circular ordering graph corresponding to i^* and j^*. Add the edge (u,v) to the circular ordering graph and coarsen the partition $\mathcal{C}$ by merging C_{r^*} and C_{s^*}.
  [**Adjustment step**] Adjust $\mu(i), i \in C_{r^*} \cup C_{s^*}$ so that $\sum_{i \in C_{r^*} \cup C_{s^*}} \mu(i) = 1$.
  [**Tree construction step**] Add the split $\{\{C_{r^*} \cup C_{s^*}\}, \{\cup_{t \neq r^*, s^*} C_t\}\}$ to the distinguished list.
end
Output the circular ordering π and the split system $\mathcal{T}$.

Definition 12. Let $\mu : X \to \mathbb{R}$ be a weighting for a partial circular ordering $\mathcal{C}$, and consider a new weighting $\mu' : X \to \mathbb{R}$ for the adjustment step of neighbor-net. μ' is a *tree weighting* if it satisfies

$$\mu'(i) = \begin{cases} \alpha\mu(i) & \text{if } i \in C_r, \\ (1-\alpha)\mu(i) & \text{if } i \in C_s, \end{cases}$$

where C_r and C_s are the two blocks being merged in the merging step and $0 \leq \alpha \leq 1$.

Tree weightings are so named because of the following proposition:

Proposition 13. *The split system $\mathcal{S}$ output by neighbor-net on input δ is pairwise compatible, and in bijection with a binary tree T. If μ is a tree weighting then the tree T is the neighbor joining tree for δ, where the agglomeration parameter at every step is given by the tree weighting parameter α.*

Proof: Note that the addition of an edge to the graph G during a run of the algorithm results in a coarsening of the partition $\mathcal{C}$, where two blocks are merged into one. For this reason, if $S_1 = \{A_1, B_1\}$ is a split added before $S_2 = \{A_2, B_2\}$ to $\mathcal{S}$, then either

$A_1 \cap A_2 = \emptyset$ or $A_1 \cap B_2 = \emptyset$. To see that the tree determined by $\mathcal{T}$ is the neighbor-joining tree, it suffices to note that selection step 1, together with the adjustment step specified by a tree weighting, is identical to the agglomeration procedure of neighbor-joining. With a tree weighting, selection step 2 and the fixed ordering within clusters has no effect on the adjustment or tree construction steps. If we simply omit the selection step 2 and the merge step, the neighbor-net algorithm reduces to neighbor-joining. □

Proposition 13 justifies the term *tree construction step* in the neighbor-net algorithm and shows that the output of neighbor-net is not only a circular ordering, but also a tree. The connection to the neighbor-joining tree is explored further in Section 5.

The coarsenings of the partition $\mathcal{C}$ in the merge step are also closely related to *graph tubings* [22]:

Definition 14. Let G be a finite graph. A *tube* is a proper nonempty set of vertices whose induced graph is a proper, connected subgraph of G. A pair of tubes r, s are *nested* if $r \subset s$ or $s \subset r$. They *intersect* if they are not nested and $r \cap s \neq \emptyset$, and two tubes are *adjacent* if $r \cap s = \emptyset$ and $r \cup s$ is a tube. Two tubes are *compatible* if they do not intersect and are not adjacent. A *tubing* of G is a set of tubes that are pairwise compatible.

Proposition 15. *Let P_{n-1} be the path on $n-1$ vertices. A labeling of P_{n-1} is a bijection from $\{1, \ldots, n-1\}$ to P_{n-1}. The output of neighbor-net is a labeling of P_{n-1} together with a maximal tubing of its line graph $L(P_{n-1})$.*

Proof: Each coarsening of $\mathcal{C}$ corresponds to a tube in $L(P_{n-1})$.

Definition 16 ([12])**.** For a graph G with n vertices, the graph-associahedron $\mathcal{P}G$ is the convex polytope of dimension $n-1$ whose face poset is isomorphic to the set of valid tubings of G, with the poset order corresponding to nesting of tubes.

The *associahedron* (denoted by K_n) refers to the graph-associahedron of the path P_{n-1}, and its vertices are in bijection with tubings of the path.

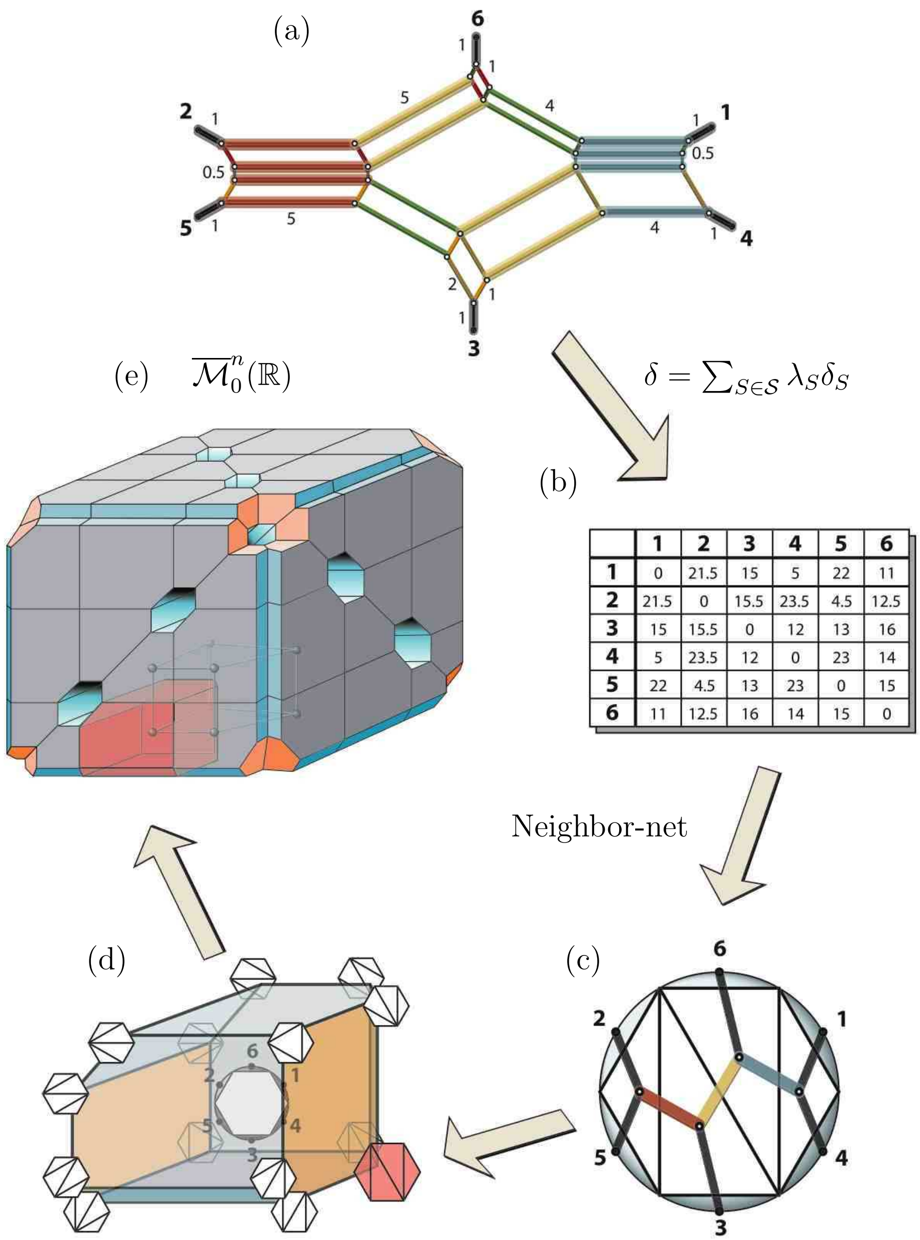
Proposition 17 (See Figure 1(c,d))**.** *The number of vertices of K_{n-1} is given by the Catalan number $\frac{1}{n-1}\binom{2n-4}{n-2}$. The vertices are in bijection with tubings of the path P_{n-2}, triangulations of the convex n-gon, and rooted binary trees with $n-1$ leaves.*

We have listed just a few of the objects in bijection with the vertices of K_n. In fact, there are dozens of combinatorial objects enumerated by the Catalan numbers (see [46]. In the context of the neighbor-joining algorithm, Proposition 17 appears as Proposition 3.1(ii) in [45].

Proposition 17 allows us to enumerate the total number of possible outputs of the neighbor-net algorithm.

Proposition 18. *The number of possible outputs of neighbor-net for n taxa is*

$$\frac{(2n-5)!}{(n-3)!}.$$



	1	2	3	4	5	6
1	0	21.5	15	5	22	11
2	21.5	0	15.5	23.5	4.5	12.5
3	15	15.5	0	12	13	16
4	5	23.5	12	0	23	14
5	22	4.5	13	23	0	15
6	11	12.5	16	14	15	0

FIGURE 1. (a) A split network representation of a circularly decomposable metric. Each split S corresponds to a color class with the length of the edges in the class λ_S indicating the size of the split. (b) The metric δ derived from the splits network. (c) The output of neighbor-net on input δ. The tree is the neighbor-joining tree. Note that its edges are highlighted in the splits network. (d) The associahedron K_5 corresponding to the circular ordering $\pi = \{1, 4, 3, 5, 2, 6\}$ and the vertex corresponding to the neighbor-joining tree. (e) The space of phylogenetic networks $\overline{\mathcal{M}}_0^n(\mathbb{R})$.

Proof: The number of distinct circular orderings (where two orderings are equivalent under the action of the dihedral group) is $\frac{1}{2}(n-1)!$ so the total number of possible outputs is

$$\frac{1}{n-1}\binom{2n-4}{n-2}\cdot\frac{1}{2}(n-1)! = \frac{(2n-5)!}{(n-3)!}. \tag{3}$$

□

The first numbers are $1, 1, 1, 6, 60, 840, 15120, 332640, 8648640, 259459200, \ldots$ These numbers also appear in another context in computational biology; in genome assembly they are the number of ways that n distinguishable equal-length clones can be interleaved to form one island [40].

Propositions 15 and 17 together establish that the output of neighbor-net is a circular ordering together with the vertex of an associahedron. Equivalently, it is a labeled convex n-gon together with a triangulation. Thus, it is natural to consider $\frac{1}{2}(n-1)!$ associahedra corresponding to the distinct circular orderings. These associahedra can be glued together in a natural way so that faces are identified when the associated subdivisions of the n-gon differ by twists along the diagonal [22]. This identification corresponds exactly to the tessellation of a certain space known as $\overline{\mathcal{M}}_0^n(\mathbb{R})$ by associahedra. The space $\overline{\mathcal{M}}_0^n(\mathbb{R})$ consists of the real points of the Deligne-Knudsen-Mumford compactification of the moduli space $\mathcal{M}_0^n$ of Riemannian spheres with n labeled punctures. Its tessellation by associahedra is described in [22]. Figure 1(e) shows the example for $n = 6$. One element from the dual tessellation by $n - 3 = 3$-dimensional cubes is also shown. Each cube is divided into 8 octants, and these octants are in bijection with the possible outputs of neighbor-net (by Proposition 18 there are 840 of them). This is summarized as follows:

Remark 19. Neighbor-net is an efficient evaluation of a function from dissimilarity maps to octants in the dual tessellation by cubes of $\overline{\mathcal{M}}_0^n(\mathbb{R})$. The vertices of the cube (or equivalently, each associahedron) can be interpreted as providing the basis for circular decomposable metrics (networks) together with tubings of the path that are in bijection with trees (phylogenies). We therefore refer to $\overline{\mathcal{M}}_0^n(\mathbb{R})$ (or its dual tiling) as the *space of phylogenetic networks*[2].

We note that the relevance of $\overline{\mathcal{M}}_0^n(\mathbb{R})$ to phylogenetics was already mentioned in [6], however in that paper it was deemed unsuitable for describing the space of trees, and replaced with a quotient space equivalent to the tropical Grassmanian [41]. It is interesting that $\overline{\mathcal{M}}_0^n(\mathbb{R})$ also appears in the study of genome rearrangements [5]. It should be interesting to explore extensions of neighbor-net that produce, via agglomeration, tubings of line graphs other than P_{n-1}, thus leading to more general phylogenetic networks connected to graph associahedra.

We conclude this section by noting that our description of neighbor-net has been based on an interpretation of the algorithm as producing only combinatorial output, i.e., a circular ordering π together with a tree. In practice, it is possible to obtain

[2]The term *phylogenetic network* is also used to denote other objects, e.g. see [39].

weights λ_S for the splits in the circular split system $\mathcal{S}$ compatible with π in the course of the algorithm. This is done by setting

$$\lambda_S = \frac{1}{2}\left(\delta(x_i, x_j) + \delta(x_{i-1}, x_{j-1}) - \delta(x_i, x_{j-1}) - \delta(x_{i-1}, x_j)\right). \quad (4)$$

for every split $S = \{\{x_i, \ldots, x_j\}, \{x_{j+1}, \ldots, x_{i-1}\}\}$.

The problem with such a procedure is that there is no guarantee that all the λ_S will be non-negative, and therefore the result may not be a circular decomposable metric. This may be circumvented by setting λ_S to zero if it is negative, but this solution may lead to inaccurate results. For these reasons, a preferable procedure is to use the circular ordering π to subsequently estimate the split weights using a non-negative least squares optimization method. This was done in the original neighbor-net implementation [10].

3. The computer science

In the previous section we have explained the input and output of the neighbor-net algorithm. In this section, we show that neighbor-net is a greedy algorithm for minimizing the (suitably defined) length of a dissimilarity map with respect to a circular ordering. We begin by extending the formulation of balanced length in [45] from trees to circular decomposable metrics.

We say that a circular ordering $\pi = \{x_1, \ldots, x_n\}$ is *consistent with* $\mathcal{C}$, if for every pair of adjacent elements i, j in some $C_l \in \mathcal{C}$ there exists a k such that $x_k = i$ and $x_{k+1} = j$. We denote the circular orderings consistent with $\mathcal{C}$ by $o(\mathcal{C})$.

Definition 20. The *balanced length of a dissimilarity map* δ with respect to a partial circular ordering $\mathcal{C}$ is defined to be

$$\begin{aligned} l(\delta, \mathcal{C}) \;&:=\; \frac{1}{|o(\mathcal{C})|} \sum_{(x_1,\ldots,x_n)\in o(\mathcal{C})} \left[\frac{1}{2}\sum_{i=1}^{n} \delta(x_i, x_{i+1})\right]. \\ &= \frac{1}{2|o(\mathcal{C})|} \sum_{(i,j)\in X} \eta_{\mathcal{C}}(i,j)\delta(i,j). \end{aligned}$$

Here $\eta_{\mathcal{C}}(i,j)$ is the number of circular orderings consistent with $\mathcal{C}$ where i is adjacent j.

Remark 21. The partial circular ordering $\mathcal{C}^* = \operatorname{argmin}_{|\mathcal{C}|=1}(l(\delta, \mathcal{C}))$ is just the shortest traveling salesman tour for the dissimilarity map δ.

We extend the notion of a balanced agglomeration scheme from neighbor joining to neighbor-net:

Definition 22. A *balanced TSP weighting* is a TSP weighting where

$$\mu(i) = \begin{cases} \frac{1}{2} & i \in \hat{C}_r,\ |\hat{C}_r| = 2, \\ 1 & i \in \hat{C}_r,\ |\hat{C}_r| = 1. \end{cases}$$

Theorem 23. *Let $\mathcal{C}$ be a partial circular ordering ($|\mathcal{C}| = m$) with a balanced TSP weighting and δ a dissimilarity map. A circular ordering $\mathcal{C}'$ of size $|\mathcal{C}'| = m-1$ that extends $\mathcal{C}$ and minimizes $l(\delta, \mathcal{C}')$ is obtained by finding a pair C_{r^*}, C_{s^*} that minimize*

$$Q_\delta(C_r, C_s) = (m-2)\delta(C_r, C_s) - \sum_{t \neq r} \delta(C_r, C_t) - \sum_{t \neq s} \delta(C_s, C_t)$$

and then adding an edge between the pair of vertices corresponding to $i^* \in C_{r^*}, j^* \in C_{s^*}$ in the circular ordering graph that minimize

$$\begin{aligned}\hat{Q}_\delta(i,j) &= (m-4+|\hat{C}_{r^*}|+|\hat{C}_{s^*}|)\delta(i,j) - \sum_{t\neq r^*,s^*} \delta(i, C_t) - \sum_{t \neq r^*, s^*} \delta(j, C_t) \\ &\quad - \sum_{k \in (C_{r^*} \cup C_{s^*}) \setminus \{i\}} \delta(i,k) - \sum_{k \in (C_{r^*} \cup C_{s^*}) \setminus \{j\}} \delta(j,k).\end{aligned}$$

Proof: Let $\mathcal{C} = \{C_1, \ldots, C_m\}$ be a partial circular ordering. A neighbor-net step consists of adding an edge to $\mathcal{C}$. This constitutes selecting two paths to join (step 1), and then deciding which of the ends of the paths to join (step 2).

Lemma 24. *The number of circular orderings consistent with $\mathcal{C}$ is*

$$|o(\mathcal{C})| = \frac{1}{2}(m-1)! \prod_{r=1}^{m} |\hat{C}_r|.$$

Let $\mathcal{C}_{r,s}$ denote all of the partial circular orderings where there is an edge between endpoints of C_r and C_s in the circular ordering graph. We say that a circular ordering is consistent with $\mathcal{C}_{r,s}$ if it is consistent with one of the partial circular orderings in $\mathcal{C}_{r,s}$. Similarly, we define $o(\mathcal{C}_{r,s})$ to constitute all circular orderings consistent with some partial circular ordering in $\mathcal{C}_{r,s}$. In the following lemma we use the notation $\overline{ij}_C$ to denote that i and j are in the same block in $C \in \mathcal{C}$, and i is adjacent to j.

Lemma 25. *The number of circular orderings consistent with $\mathcal{C}_{r,s}$ is $2|o(\mathcal{C})|/(m-1)$ and*

$$\eta_{\mathcal{C}_{r,s}}(i,j) = \begin{cases} 2|o(\mathcal{C})|/(m-1) & \text{if } \overline{ij}_C \text{ for some } C, \\ 2|o(\mathcal{C})|\mu(i)\mu(j)/(m-1) & \text{if } i \in C_r,\, j \in C_s, \\ 4|o(\mathcal{C})|\mu(i)\mu(j)/(m-1)(m-2) & \text{if } i \in C_t,\, j \in C_u,\, t \neq u,\, t,u \neq r,s, \\ 2|o(\mathcal{C})|\mu(i)\mu(j)/(m-1)(m-2) & \text{if } i \in C_r,\, j \in C_t,\, t \neq s, \\ 2|o(\mathcal{C})|\mu(x)\mu(y)/(m-1)(m-2) & \text{if } i \in C_s,\, j \in C_t,\, t \neq r, \\ 0 & \text{otherwise.} \end{cases}$$

The proof of the lemma is elementary. We note that it also makes sense for weightings that are not balanced TSP weightings, except that the effect of the weightings μ is to alter the η so that they count the number of circular orderings consistent with split systems larger than $\mathcal{C}_{r,s}$. For example, if μ is a tree weighting, then η counts the number of circular orderings consistent with the partially resolved tree $\mathcal{T}$. For more on this see Definition 38 and Theorem 39.

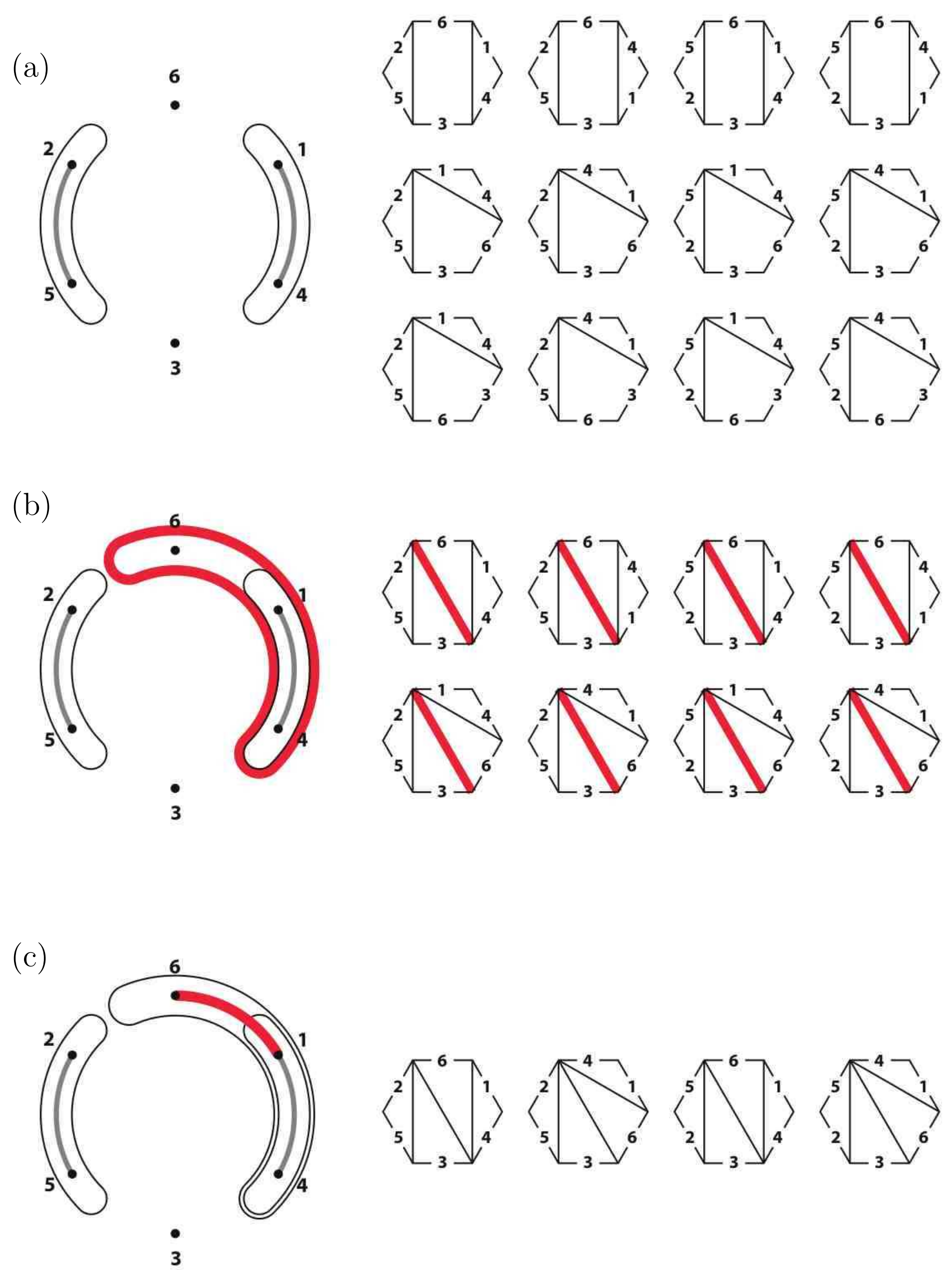


FIGURE 2. A step in the neighbor-net algorithm run on the dissimilarity map δ from Figure 1(b). (a) A partial circular ordering $\mathcal{C}$, $|\mathcal{C}| = 4$ and the 12 circular orderings consistent with it. Note that at this stage $l(\delta, \mathcal{C}) = \frac{1708}{48}$ (b) Selection step part 1 showing $\mathcal{C}_{r^*,s^*}$ where $C_{r^*} = \{6\}$ and $C_{s^*} = \{1, 4\}$. Now $l(\delta, \mathcal{C}_{r^*,s^*}) = \frac{1659}{48}$ and this is a neighbor-joining agglomeration. (c) Selection step part 2 results in a new partial circular ordering $\mathcal{C}'$, $|\mathcal{C}'| = 3|$ with 6 adjacent to 1 and $l(\delta, \mathcal{C}') = \frac{1614}{48}$. This last step is what distinguishes neighbor-net from neighbor-joining.

We can now conclude the proof of Theorem 23:

$$\begin{aligned}
l(\delta,\mathcal{C}_{r,s}) &= \frac{1}{2}\sum_{C\in\mathcal{C}}\sum_{\overline{ij}_C}\delta(i,j)+\frac{1}{2}\delta(C_r,C_s)+\frac{1}{(m-2)}\sum_{C_t\neq C_u,t,u\neq r,s}\delta(C_t,C_u)\\
&\quad+\frac{1}{2(m-2)}\sum_{C_t\neq C_r,C_s}\delta(C_t,C_s)+\frac{1}{2(m-2)}\sum_{C_t\neq C_r,C_s}\delta(C_t,C_r)\\
&= \frac{1}{2}\sum_{C\in\mathcal{C}}\sum_{\overline{ij}_C}\delta(i,j)+\frac{1}{(m-2)}\sum_{C_t\neq C_u}\delta(C_t,C_u)\\
&\quad+\frac{1}{2}\delta(C_r,C_s)-\frac{1}{2(m-2)}\sum_{C_t\neq C_s}\delta(C_t,C_s)-\frac{1}{2(m-2)}\sum_{C_t\neq C_{r^*}}\delta(C_t,C_s).
\end{aligned}$$

Thus, $l(D,\mathcal{C}_{r,s})=\frac{1}{2(m-2)}Q_\delta(C_r,C_s)+T$ where T does not depend on r or s. In other words, at each step neighbor-net is selecting a pair (r^*,s^*) to join that will minimize the balanced length. The actual minimum balanced length is attained for one of the $|\hat{C}_{r^*}||\hat{C}_{s^*}|$ possibilities for adding an edge between C_{r^*} and C_{s^*} in $\mathcal{C}$. Using the same argument as above, it is easy to see that the minimum balanced length is attained when $\hat{Q}_\delta(i,j)$ is subsequently minimized. □

Remark 26. Let

$$Z_\delta(C_r,C_s)=-\frac{1}{m-1}\sum_{C\neq C_s}\delta(C_r,C_s)-\frac{1}{2}Q_\delta(C_r,C_s).$$

Then

$$l(\delta,\mathcal{C})=\frac{1}{2}\sum_{C\in\mathcal{C}}\sum_{i,j\in C}\delta(i,j)+\frac{1}{(m-1)}T$$

implies that

$$l(\delta,\mathcal{C})-l(\delta,\mathcal{C}_{r,s})=Z_\delta(C_r,C_s).$$

The quantity $Z_\delta(C_r,C_s)$ features prominently in [16, 29, 38] and is based on the "neighborliness measurement" of [29]:

$$Z_\delta(C_r,C_s)=\sum_{t,u\neq r,s}w(C_rC_s:C_tC_u),\ \text{where}$$

$$w(C_rC_s:C_tC_u)=\frac{1}{2}(\delta(C_r,C_t)+\delta(C_r,C_u)+\delta(C_s,C_t)+\delta(C_s,C_u)-2\delta(C_r,C_s)-2\delta(C_t,C_u)).$$

It is interesting to note that the results in [38] are motivated by this alternative formulation of the neighbor-joining criterion. Remark 26 provides further evidence that the "Z-criterion" is a natural formulation for the neighbor-joining criterion, and at the same time explains the meaning of $Z_\delta(C_r,C_s)$ in terms of the balanced length.

Returning to Remark 21, we have the following interpretation of Theorem 23:

Remark 27. Neighbor-net with a balanced TSP weighting is a greedy algorithm for the traveling salesman problem.

In fact, neighbor-net provides the optimal solution for the TSP when δ satisfies the Kalmanson conditions (see Theorem 29 in Section 4). It is well known that the TSP can be solved in polynomial time $O(n^2 \log n)$ for Kalmanson matrices [17]; neighbor-net provides an alternative $O(n^3)$ polynomial algorithm. The $O(n^3)$ running time is based on the observation that the TSP and tree weighting schemes can be implemented so that the selection steps are $O(k^2)$ where k is the number of blocks in the partial circular ordering at each step. It should be possible to obtain further improvements in speed by using the ideas developed for fast neighbor-joining [27].

Theorem 23 is restricted to the balanced TSP weighting. We note, however, that there is no practical limitation to using different weightings for the first and second selection steps. We may consider a hybrid algorithm that applies a tree weighting to the first selection step and a balanced TSP weighting to the second. In that case, Proposition 13 together with Theorem 23 show that

Remark 28. Neighbor-net with a hybrid weighting scheme is a greedy algorithm for finding, simultaneously, the tree of minimum balanced length and the circular ordering of minimum length consistent with it.

4. The statistics

We begin in this section by showing that neighbor-net is a robust algorithm. By this we mean that if the input to neighbor-net is a dissimilarity map δ that is a perturbation of a circular decomposable metric with respect to a circular ordering π, neighbor-net outputs the circular ordering π. We note that in the case of a circular decomposable metric where some of the splits have zero weight, there will be more than one circular ordering consistent with δ. In that case neighbor-net will output one of those circular orderings. A corollary to this is that if δ is a circular decomposable metric, and equation (4) is used to estimate the distances, then the output is exactly δ, i.e., neighbor-net is a consistent estimator of the parameters of a circular decomposable metric. Implicit in the neighbor-net estimator are assumptions about the variances of the measured distances. These can be interpreted in terms of the weighting scheme used in neighbor-net, and we return to this at the end of the section.

Theorem 29. *Suppose that $\delta : X \times X \to \mathbb{R}$ is a dissimilarity map that satisfies the Kalmanson conditions for some circular ordering π. Then neighbor-net applied to δ outputs a circular ordering π' such that $W_\delta \subseteq W_{\pi'}$.*

Proof: It suffices to show that at any step of the algorithm, every circular ordering consistent with the partial circular ordering contains all the quartets in W_δ. Let $\mathcal{C} = \{C_1, \ldots, C_m\}$ be a partial circular ordering consistent with π so that if $x_i \in C_r$ and $x_j \in C_s$ and $r < s$ then $i < j$.

Lemma 30. *For every $r < s < t < u$,*

$$\begin{aligned} \delta(C_r, C_s) + \delta(C_t, C_u) &\leq \delta(C_r, C_t) + \delta(C_s, C_u) \\ \delta(C_r, C_u) + \delta(C_s, C_t) &\leq \delta(C_r, C_t) + \delta(C_s, C_u). \end{aligned}$$

Proof: This follows directly from the Kalmanson conditions and the requirement that $\sum_{i \in C_r} \mu(i) = 1$ for every r. □

Moreover, if for some $a \in C_r, b \in C_s, x \in C_t, y \in C_u$ with $\mu(a), \mu(b), \mu(x), \mu(y) > 0$ we have $(ab; xy) \in W_\delta$, then $\delta(C_r, C_s) + \delta(C_t, C_u) < \delta(C_r, C_t) + \delta(C_s, C_u)$.

Next we introduce some notation to simplify the necessary calculations. We set $\delta_{C_r C_s}(C_t) = \delta(C_r, C_t) + \delta(C_s, C_t) - \delta(C_r, C_s)$. This is an analog of the *Farris transform* [28] for blocks in the partial circular ordering $\mathcal{C}$. Note that

$$Q_\delta(C_r, C_s) = -2\delta(C_r, C_s) - \sum_{C_t} \delta_{C_r C_s}(C_t). \tag{5}$$

In order to simplify the presentation, we replace every C_i with i in the formulas below. This is mathematically justified by Lemma 30 since blocks in a partial circular ordering behave exactly like elements of the underlying set X with respect to the Kalmanson conditions. For example, by $Q_\delta(i, i+1)$ in the lemma below, we mean $Q_\delta(C_i, C_{i+1})$ and a proof that $Q_\delta(C_i, C_{i+2}) > Q_\delta(C_i, C_{i+1})$ is equivalent to the proof that $Q_\delta(i, i+2) > Q_\delta(i, i+1)$ by Lemma 30.

Lemma 31.

$$Q_\delta(i, i+2) - Q_\delta(i, i+1) \geq 0.$$

Proof: Let $j = i+2$, $k = i+1$.

$$Q_\delta(i, j) - Q_\delta(i, k) = \sum_{x \neq i,j,k} \delta(k, x) + \delta(i, j) - \delta(i, k) - \delta(j, x)$$

and $\delta(k, x) + \delta(i, j) - \delta(i, k) - \delta(j, x) \geq 0$ for each x by Lemma 30. □

Lemma 32 (The Anarchy Lemma)**.**

$$Q_\delta(i, i+3) - Q_\delta(i+1, i+2) \geq 0.$$

Proof: Let $j = i+3$, $k = i+1$, $l = i+2$. Applying Lemma 30 twice:

$$\begin{aligned} Q_\delta(i, j) - Q_\delta(k, l) &= \sum_{x \neq i,j,k,l} (\delta(i, j) + \delta(k, x) + \delta(l, x)) - \delta(i, x) - \delta(j, x) - \delta(k, l) \\ &\geq \sum_{x \neq i,j,k,l} (\delta(j, k) + \delta(i, x) + \delta(l, x) - \delta(i, x) - \delta(j, x) - \delta(k, l)) \\ &\geq \sum_{x \neq i,j,k,l} (\delta(j, k) + \delta(l, x) - \delta(j, x) - \delta(k, l)) \geq 0. \end{aligned}$$

□

Lemma 33. *Let $i < x < y < z < j < t$. Then*

$$\begin{aligned} & \delta_{xy}(z) + \delta_{xz}(y) + \delta_{yz}(x) + \delta_{xy}(t) + \delta_{xz}(t) + \delta_{yz}(t) \\ \geq\ & 3\delta_{ij}(t) + \delta_{ij}(x) + \delta_{ij}(y) + \delta_{ij}(z). \end{aligned}$$

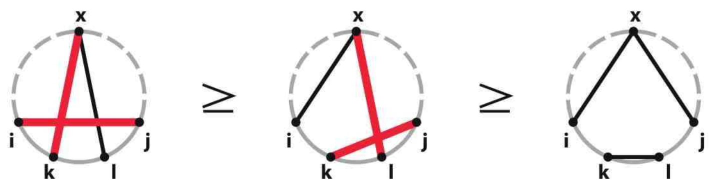


FIGURE 3. An illustration of the proof of Lemma 32.

Proof: Note that each of the following inequalities follows directly from Lemma 30:

$$\begin{aligned}2\delta(x,t)+2\delta(i,j) &\geq \delta(x,i)+\delta(x,j)+\delta(i,t)+\delta(j,t)\\ 2\delta(y,t)+2\delta(i,j) &\geq \delta(y,i)+\delta(y,j)+\delta(i,t)+\delta(j,t)\\ 2\delta(z,t)+2\delta(i,j) &\geq \delta(z,i)+\delta(z,j)+\delta(i,t)+\delta(j,t).\end{aligned}$$

Summing both sides we obtain the required inequality. □

Proposition 34. *Suppose that $i < j-3$. Then there exists k such that*

$$Q_\delta(i,j) - Q_\delta(k,k+1) \geq 0. \tag{6}$$

Proof: Recall that $|\mathcal{C}| = m$. Suppose without loss of generality that $i = 0$ and $j \leq m/2$. We will find $i < k \leq j-2$ satisfying (6), where the proof is non-constructive and mimics the arguments in Theorem 25 of [38]. In particular, we show that

$$(j-3)\sum_{0<x,y<j}(Q_\delta(i,j) - Q_\delta(x,y)) \geq 0, \tag{7}$$

so that there exists $i < x, y < j$ with $Q_\delta(i,j) - Q_\delta(x,y) \geq 0$.

We first note that

$$Q_\delta(i,j) - Q_\delta(x,y) = \sum_{t\neq i,j,x,y} \delta_{xy}(t) - \delta_{ij}(t).$$

We then break this sum into three sections: those that fall between 0 and j, a matching set of the same size $(j-3)$ that lie beyond j, and lastly, all remaining terms. In this way, equation (7) equals

$$(j-3)\sum_{0<x,y<j}\left(\sum_{\substack{z=1\\ z\neq x,y}}^{j-1}\delta_{xy}(z)-\delta_{ij}(z)+\sum_{t=j+1}^{2j-3}\delta_{xy}(t)-\delta_{ij}(t)+\sum_{s=2j-3}^{m-1}\delta_{xy}(s)-\delta_{ij}(s)\right)$$

By Lemma 32, the last summation is greater than or equal to zero, and so:

$$
\begin{aligned}
&\geq \sum_{0<x,y<j} (j-3) \left(\sum_{\substack{z=1 \\ z \neq x,y}}^{j-1} \delta_{xy}(z) - \delta_{ij}(z) + \sum_{t=j+1}^{2j-3} \delta_{xy}(t) - \delta_{ij}(t) \right) \\
&= \sum_{0<x,y<j} \sum_{\substack{z=1 \\ z \neq x,y}}^{j-1} \sum_{t=j+1}^{2j-3} \delta_{xy}(z) + \delta_{xy}(t) - \delta_{ij}(z) - \delta_{ij}(t) \\
&= \sum_{t=j+1}^{2j-3} \sum_{\substack{0<x,y,z<j, \\ x \neq y \neq z}} \delta_{xy}(z) + \delta_{xz}(y) + \delta_{yz}(x) + \delta_{xy}(t) + \delta_{xz}(t) + \delta_{yz}(t) \\
&\qquad -3\delta_{ij}(t) - \delta_{ij}(x) - \delta_{ij}(y) - \delta_{ij}(z) \quad \geq \quad 0.
\end{aligned}
$$

The final inequality follows from Lemma 33. The claim (6) now follows by noting that repeated application of the argument leads to one of three cases: either we find a pair of neighbors $k, k+1$ such that $Q_\delta(k,k+1) \leq Q_\delta(i,j)$, or else we find a pair that are separated by one node (in which case we apply Lemma 31) or a pair that are separated by two nodes (in which case we apply Lemma 32). □

Returning to the proof of the theorem, it is clear that if we have a strict Kalmanson inequality on any quartet that separates i and j, then the inequalities in Lemmas 31, 32 and Proposition 34 are strict inequalities. Consequently we never join a pair of blocks that violate a quartet in W_δ. If the blocks are of size 1 we are done. Otherwise, it only remains to show that two neighboring elements $x_r \in C_i$ and $x_{r+1} \in C_{i+1}$ will be selected to be joined in the minimization of $\hat{Q}$. This follows directly from the same arguments used in Lemmas 31 and 32. □

The consistency of neighbor-net now follows easily by observing that for a circular decomposable metric, the distances will be correctly inferred using (4).

Corollary 35 ([9]). *Neighbor-net is statistically consistent.*

Moreover, Theorem 29 can be used to obtain a neighbor-net analog of Atteson's theorem [2] on the optimal radius of neighbor-joining:

Corollary 36 (Optimal radius). *Let $\mathcal{S}$ be a circular split system with respect to a circular ordering $\pi = \{x_1, \ldots, x_n\}$, $\lambda_S > 0$ for every $S \in \mathcal{S}$, and $\delta_{\mathcal{S}} = \sum_{S \in \mathcal{S}} \lambda_S \delta_S$ a circular decomposable metric. If $\epsilon = min_{S \in \mathcal{S}} \lambda_S$ and δ is any dissimilarity map with $||\delta - \delta_{\mathcal{S}}||_\infty < \frac{\epsilon}{2}$ then neighbor-net will output a circular ordering whose split system contains $\mathcal{S}$.*

Proof: It suffices to show that if $||\delta - \delta_{\mathcal{S}}||_\infty \leq \frac{\epsilon}{2}$ then δ satisfies the Kalmanson conditions with respect to π. Let $i < j < k < l$.

$$\delta_{\mathcal{S}}(x_i, x_k) + \delta_{\mathcal{S}}(x_j, x_l) - \delta_{\mathcal{S}}(x_i, x_j) - \delta_{\mathcal{S}}(x_k, x_l) = \sum_{S = \{A,B\}, i,j \in A, k,l \in B} 2\lambda_S.$$

Therefore,

$$\delta(x_i,x_k)+\delta(x_j,x_l)-\delta(x_i,x_j)-\delta(x_k,x_l) \geq \left(\sum_{S=\{A,B\},i,j\in A,k,l\in B} 2\lambda_S\right) - 2\epsilon > 0.$$

A similar argument shows that $\delta(x_i,x_k)+\delta(x_j,x_l)-\delta(x_j,x_k)-\delta(x_k,x_i) \geq 0$. □

Note that in Corollary 36 the dissimilarity map δ satisfying $||\delta-\delta_{\mathcal{S}}||_\infty \leq \frac{\epsilon}{2}$ may not be a metric. Kalmanson matrices (as opposed to metrics) are characterized in [18].

We have already hinted at connections between neighbor-net and the traveling salesman problem in Section 3. Our next theorem demonstrates the consistency of the TSP estimate of the circular ordering and is analogous to Theorem 2 of [20].

Theorem 37. *Let δ be a generic circular decomposable metric with respect to a circular ordering $\pi = \{x_1,\ldots,x_n\}$. Then $l(\delta,\sigma) > l(\delta,\pi)$ for any circular permutation $\sigma = \{y_1,\ldots,y_n\}$ different from π.*

Proof: Since δ is a circular decomposable metric it must satisfy the Kalmanson conditions. Therefore there must exist $i<k$, $|k-i|>1$ such that $\delta(y_i,y_{i+1})+\delta(y_k,y_{k+1}) > \delta(y_i,y_k)+\delta(y_{i+1},y_{k+1})$. Consider the circular ordering

$$\sigma' = \{y_1,\ldots,y_i,y_k,y_{k-1},\ldots,y_{i+1},y_{k+1},y_{k+2},\ldots,y_n\}.$$

Then $l(\delta,\sigma') < l(\delta,\sigma)$ and therefore $\text{argmin}_\tau l(\delta,\tau)=\pi$. □

This result explains why it makes sense to use TSP solutions directly for finding circular orderings [36].

We now turn to the statistical meaning of the weighting μ in the neighbor-net algorithm, and discuss how it should be chosen in practice. We first consider the case of tree weightings. In this case neighbor-net outputs a circular ordering consistent with the neighbor-joining tree (Proposition 13). The theory of [20] together with our results provides a direct interpretation of the agglomeration parameters that can be summarized as follows:

Definition 38 (Length of a split system)**.** Let $\mathcal{S}$ be a split system that is circular with respect to some circular ordering and let $\eta_{\mathcal{S}}(i,j)$ be the number of circular orderings consistent with $\mathcal{S}$ where where x is adjacent to y. The length of a dissimilarity map δ with respect to $\mathcal{S}$ is

$$l(\delta,\mathcal{S}) = \sum_{i,j} \eta_{\mathcal{S}}(i,j)\delta(i,j).$$

Theorem 39. *Let δ be a dissimilarity map, $\mathcal{S}$ a split system that is circular with respect to some circular ordering, and $\eta_{\mathcal{S}}(i,j)$ defined as above. Let $\delta^* = \sum_{S\in\mathcal{S}}\lambda_S\delta_S$ ($\lambda_S \geq 0$) be the circular decomposable metric obtained from the weighted least squares estimates of the splits under the assumption that the variance of $\delta(i,j)$ is $\kappa\eta_{\mathcal{S}}(i,j)^{-1}$ (with the same constant κ for all i,j). Then*

$$l(\delta,\mathcal{S}) = \sum_{S\in\mathcal{S}}\lambda_S.$$

The choices of agglomeration parameters for a tree weighting determine $\eta_{\mathcal{S}}(i,j)$ at each step and are therefore implicit variance assumptions on the distances for the weighted least squares tree that is being greedily approximated by the algorithm. The balanced tree weighting scheme for neighbor-net corresponds to balanced neighbor-joining agglomeration [20]. It should be interesting to explore `BIONJ` [30] analogs for neighbor-net, which is easy to do since it only involves adapting the tree weightings. In the case of a balanced TSP weighting, Theorem 39 explains that the neighbor-net algorithm ignores nodes once they have two neighbors after agglomeration.

We conclude by remarking that some progress has been made in the development of statistical models for split networks, suggesting the possibility for maximum likelihood approaches to finding circular split systems [7, 47].

5. Applications

Our goal in this section is to show how the theorems proved in the previous sections provide insight into how to use neighbor-net in practice, and in how to infer split networks. We begin with an observation regarding the distance reduction formula used in the current implementations of neighbor-net.

The agglomeration scheme proposed in [10] is as follows: Suppose that a circular ordering contains two blocks C_r, C_s that are being agglomerated, where C_r is a union of two smaller blocks $C_r = C_t \cup C_u$ so that the agglomerated block is $C_r \cup C_s = C_t \cup C_u \cup C_s$ in that order.

$$\mu'(i) = \begin{cases} \frac{1}{4}\mu(i) & i \in C_t \cup C_s, \\ \frac{1}{2}\mu(i) & i \in C_u. \end{cases} \tag{8}$$

There is an analogous formula for the case when two blocks, each composed of two blocks are being joined (the above formula is applied twice).

This weighting is neither a TSP weighting nor a tree weighting. Furthermore, in the case of agglomeration of a pair of blocks each composed of two blocks, the resulting weighting μ depends on the order in which the agglomeration is performed. Thus, the tree output by neighbor-net using (8) is not necessarily the neighbor-joining tree, whereas the use of a tree-weighting scheme guarantees this (Proposition 13).

The advantage of producing a circular ordering consistent with the neighbor-joining tree, is that it allows for a direct analysis of the conflicting signals with a tree of interest. To demonstrate this, we analyzed a published dataset of language structure characters from Oceanic Austronesian and Papuan languages [26]. The neighbor-net algorithm was previously used to infer phylogenetic relationships among the languages (Figure S2 from the supplementary materials of [26]). We compared Figure S2 obtained using the default parameters for neighbor-net (8) with the balanced tree weighting scheme that produces a neighbor-joining tree. In both cases, the split weights were computed using the constrained least squares estimation procedure in [10]. The split networks were visualized using the program `SplitsTree4` [33]. Figure 4(a) shows the network for the balanced tree weighting scheme, together with the neighbor-joining tree corresponding to the split system output by the algorithm. The circular ordering obtained by using the

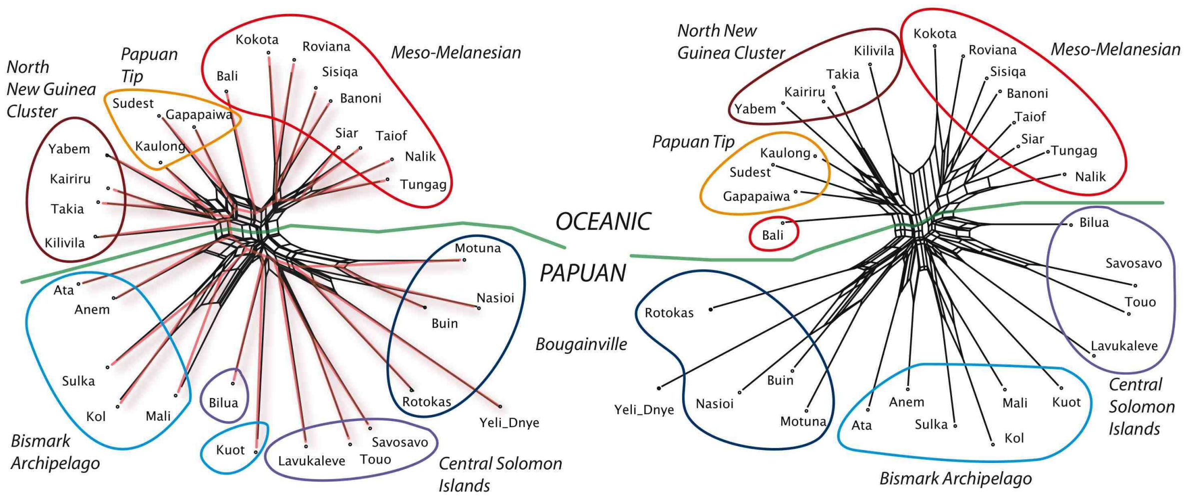


Figure 4. Left: Neighbor-net and the neighbor joining tree for groups of Papuan and Austronesian languages. Right: The split-network inferred for the optimal circular ordering that was obtained using `Concorde`.

default neighbor-net settings is not consistent with this neighbor-joining tree. The ability to view the neighbor-joining tree in conjunction with the neighbor-net split network is a direct result of Proposition 13. The representation of the tree together with the network, as shown in Figure 4(left), is useful for directly using neighbor-net to evaluate the extent of phylogenetic discordancy with the neighbor-joining tree. For example, we see clearly that the split between the Papuan and Austronesian (Oceanic) languages is in fact a split in the neighbor-joining tree. Note that all the edges in the network and tree are drawn to scale.

The interpretation of neighbor-net as a greedy algorithm for the TSP suggests an analysis of the optimal TSP tour. We computed this tour for the dataset from [26] using `Concorde` [1]. The optimal tour, of length 7.541 was found in 0.57 seconds. The length of this tour should be contrasted with the length of the balanced tree weighting tour, 7.810, which is very close to 7.794, the length of the tour obtained using the default parameters. The constrained least squares optimization procedure of [10] was applied to the optimal circular ordering and resulted in the split network shown in Figure 4(right).

The comparison of the two split networks in Figure 4 is interesting. A key observation in [26] was that the Papuan languages cluster into groups consistent with the geographical locations of the islands. On the other hand, it was remarked that Bougainville, which is geographically in between the Bismarck Archipelago and the Central Solomon Islands, did not cluster in between the languages from those two locations. Figure 4(right) shows that the TSP ordering produces a better overall clustering, albeit still with the Bougainville languages not sandwiched in the geographically correct location. Nevertheless, a key new insight that emerges from the network is that Bali, which appears to be incorrectly grouped, is in fact correctly grouped if one assumes that the

Papuan and Oceanic groups are really two distinct separate groups (it is then just a neighbor to Nalik).

Our main conclusion is that the choice of weightings in the neighbor-net algorithm is important in determining the results, and that care has to be taken in choosing the weights appropriately. Furthermore, tree weighting algorithms will be useful in cases where it is desirable to use neighbor-net as a diagnostic tool for exploring neighbor-joining trees, and TSP algorithms may be useful for direct application in obtaining circular orderings. In fact, the use of TSP solvers in similar contexts is not new, appearing in [36] in the context of tree construction and in [34], where the `Concorde` program is used to find a circular ordering from a distance matrix for proteins based on protein-protein interactions. It also seems important to develop a variant of neighbor-net that outputs the optimal circular ordering consistent with an arbitrary given tree.

We conclude by noting that neighbor-net can also be used practically as a greedy algorithm for the TSP. Unlike the naive greedy algorithm for which many negative results have been published (see, e.g., [4]), neighbor-net exhibits good properties. For example, the output does not depend on the order of the input, and the algorithm is optimal for Kalmanson matrices. We experimented with the problem st70.tsp from `TSPLIB` [42]. The balanced TSP weighting gave a tour of length 759.801, that is only 12% longer than the optimal tour of length 678.598. As expected, the balanced tree weighting scheme yielded a longer tour of length 812.613. It will be interesting to explore the improvements possible with the incorporation of search heuristics such as nearest neighbor interchange moves. These have been used to significantly improve neighbor-joining in the `FastME` program [19].

Acknowledgments

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