

Comparison of Two Possible Evolutionary Mechanisms of the 62 tRNA Codon Sequences

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Abstract

The tRNAs were divided into 62 groups based on the codons they carried and networks were constructed. Phylogenetic trees were constructed in parallel and antiparallel directions based on the parameters of these networks. Point mutations were found in the codon sites in the same clusters in the entire parallel and antiparallel phylogenetic trees, whereas there was no evidence of a complementary duplication mechanism in the corresponding antiparallel phylogenetic trees. The codons of isoaccepting tRNAs were found in neighboring clusters or distributed within a very small distance so the codons with only one base difference remained very close, which was perfectly consistent with the hypothesis that a new tRNA gene could be recruited from an isoaccepting group via another point mutation.

Keywords

Phylogenetic Trees, Point Mutation, Template Duplication, Network

1. Introduction

There are many reports on mitochondrial (mt) tRNA mutations would linked with inherited diseases [1] [2] [3], *i.e.*, mutations in $tRNA^{Ile}$, $tRNA^{Lys}$ and $tRNA^{Ser(UCN)}$ are known to cause different mt encephalomyopathies or non-syndromic deafness [3]. The mutation of tRNA genes has a research hotspot. Many tRNA sequences have been found and deposited in a database [4] and they all conform to one of 62 codon sequence groups (two codon groups are absent from the database, *i.e.*, aaa and cta). All modern tRNA sequences have evolved from a common ancestor, but their evolutionary mechanism remains an open question. Two possible mechanisms have been proposed to explain the origin and evolution of modern tRNAs, *i.e.*, “point mutation” and “template duplication” [5]. The “point mutation” mechanism suggests that divergence occurred mainly via

“mutation” [6] [7] [8] [9] [10]. It is hypothesized that a new sequence may be recruited from the mutant RNA molecule or be recruited from an isoaccepting group by another point mutation in the sequence that concurrently changed the tRNA amino acid identity and its messenger RNA coupling capacity, which is known as “codon capture” or “tRNA gene recruitment” [11]. In contrast to evolution via mutation, the “template duplication” mechanism proposes that tRNAs diverged from a precursor via a duplication mechanism. It is hypothesized that primordial tRNAs with hairpin structures associate themselves with a template and produce another RNA via sequence complementarity [12]-[17]. It is thought that four initial pairs of pre-tRNAs with complementary anti-codons could have been capable of generating all 64 anticodons.

Theoretically, if modern tRNAs evolved via point mutations, the matching scores of sequences that align in a parallel direction, 5' → 3' vs. 5' → 3', should be larger than the scores aligned in an antiparallel direction, 5' → 3' vs. 3' → 5'. If modern tRNAs evolve by complementary duplication method, however, the opposite would be true. To identify the most likely mechanism for tRNA evolution, we compared tRNA sequences in parallel and antiparallel directions, and we constructed two types of networks to make a comparison. The corresponding parallel and antiparallel phylogenetic trees of the 62 codon sequences could be generated by altering the parameters of these networks using the neighbor-joining method to different degrees. We discuss the evolutionary properties of the codon sequences for the 62 tRNA groups and the two types of phylogenetic trees.

2. Materials and Methods

2.1. Experimental Data

The 3695 tRNA sequences are acquired from the database (<http://trnadb.bioinf.uni-leipzig.de/>) [1] and the highly similar species in the database were filtered. Each tRNA sequence contained 76 bases and we removed the bases in the variable stem and the poly-A tail.

2.2. tRNA Network Construction

2.2.1. Important Network Parameters

Degree and clustering coefficient: the network is made up of a set of nodes (vertices) and the connections between them (edges or links). The features and the nature of the network were indicated by two main parameters [18], *i.e.*, the degree, k , and the clustering coefficient, c , of the nodes. The degree k of a node was the number of nodes with which it was connected. If a node connected with i other nodes and j edges had connections within these i nodes, the clustering coefficient c of the original node was defined as $C = 2j/i(i-1)$, where $i(i-1)/2$ is the total number of possible connections among i nodes. The clustering coefficient reflected the relationships among the neighbors of a node and it quantified the inherent tendency of the network to cluster. Investigating the degree and clustering coefficient distribution of the network facilitated the study of the local

where i and j ran from 1 to 62. In this study, L was the length of one tRNA, and N was the total number of nodes in the network. We use $\langle D(i, j) \rangle$ and $\langle C(i, j) \rangle$ to represent the alignment scores of two different codon groups, which showed the evolutionary affiliation of tRNAs with two different codons, which helped us to interpret the overall evolutionary trend of the tRNA sequences to some extent.

(iii) We constructed the phylogenetic tree for the 62 tRNA codon sequences using PHYLIP 3.67 and TreeView 1.6.6.

3. Results

Figure 2 shows the diverse relationships among the phylogenetic trees of the 62 tRNA codon groups. Each tree branch represents one group with a specific codon sequence. **Figure 2(a)** shows that a degree of similarity of $S = 10$ produced two large clusters, *i.e.*,

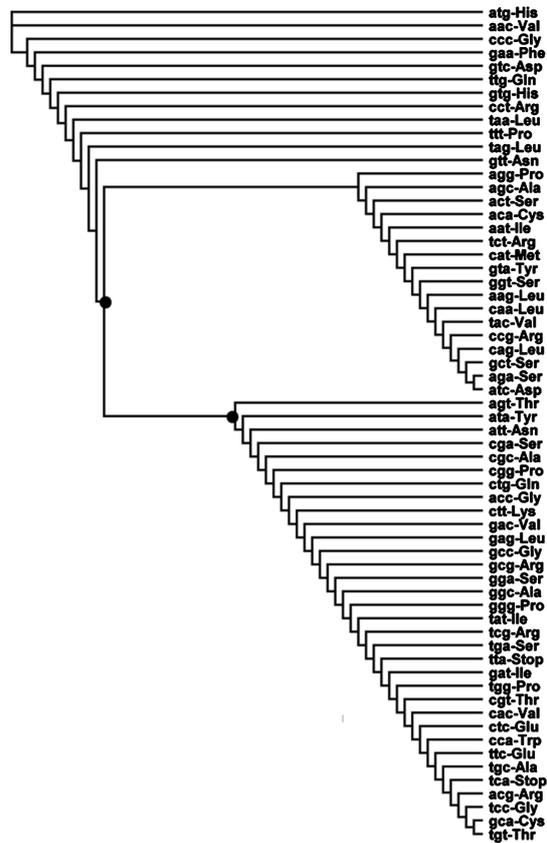
((att,(cct,(act,(tgt,(tga,(cga,(tat,(tct,(gct,(aag:agt)))))))))),
(cgg,(tca,(gca,(agg,(tcg,(atc,(acc:acg)))))), gcc,(gcg,(gtg:tgg)) and
((cgc,(gta,(gtc,(gac,(aca,(ctg,(ggg:ccc))))))),

agc,(cca,(acc,(ggt,(aga,(gga,(ctt:ggt))))),ggc,(ctc,(tcc:ttg)), as well as 15 isolated branches and one small cluster in the overall parallel phylogenetic tree, where each large cluster comprised three small clusters. the sequences atg, aac, tta, ata, caa, ttt, cat, gat, gag, tag, gaa, and taa were found in isolated branches, but their evolutionary distance was small. They exhibited a regular ladder ranked appearance. All of the codons in the large clusters supported the point mutation mechanism and each had a matching codon with only one base difference in the same site. We also found that some codons of the isoaccepting tRNAs appeared in the same cluster and neighboring clusters. For example, six codons the amino acid *Arg*, *i.e.*, cct, tct, tcg, acg, ccg, and gcg, for were in the same cluster, three of them were in a neighboring branch, and three were dispersed among different branches. In contrast to the parallel phylogenetic tree for $s = 10$, the antiparallel phylogenetic tree for $s = 10$ only contained two large clusters and some isolated branches, and the codons of these two large clusters exhibited a regular ladder rank. Thus, these results did not support “template duplication mechanism.” Some codons of isoaccepting tRNA sequences also appeared in the same cluster, *e.g.*, four codons for *Ser* were in the same cluster, whereas the other two codons were in another cluster. Most codons for isoaccepting tRNA sequences were dispersed throughout the tree.

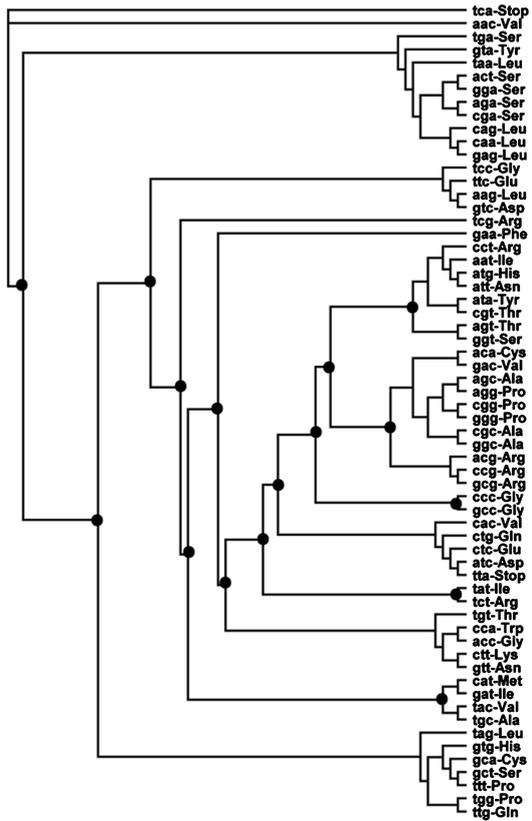
For $s = 35$, a comparison of **Figure 2(a)** and **Figure 2(c)** showed that the distribution of the parallel phylogenetic tree in **Figure 2(c)** was clearly different, with fewer isolated branches while the codons in the large cluster were more dispersed. Two small clusters and one large cluster comprised the whole tree, where most codons had matching codons with only one base difference in the small cluster, although a few had no mutated codons in the upstream branch. More codons of the isoaccepting tRNA sequences were present in the same cluster and they were closer. As shown in **Figure 2(d)**, the distribution of the anti-



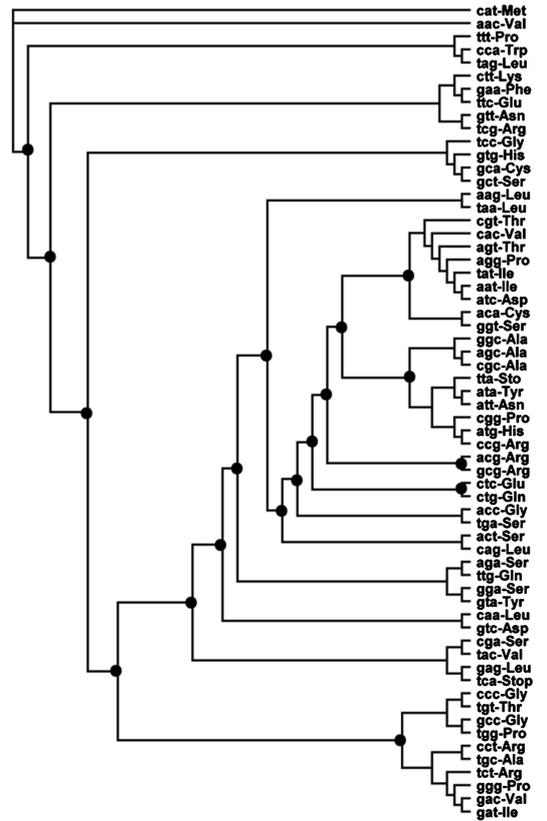
(a)



(b)



(c)



(d)

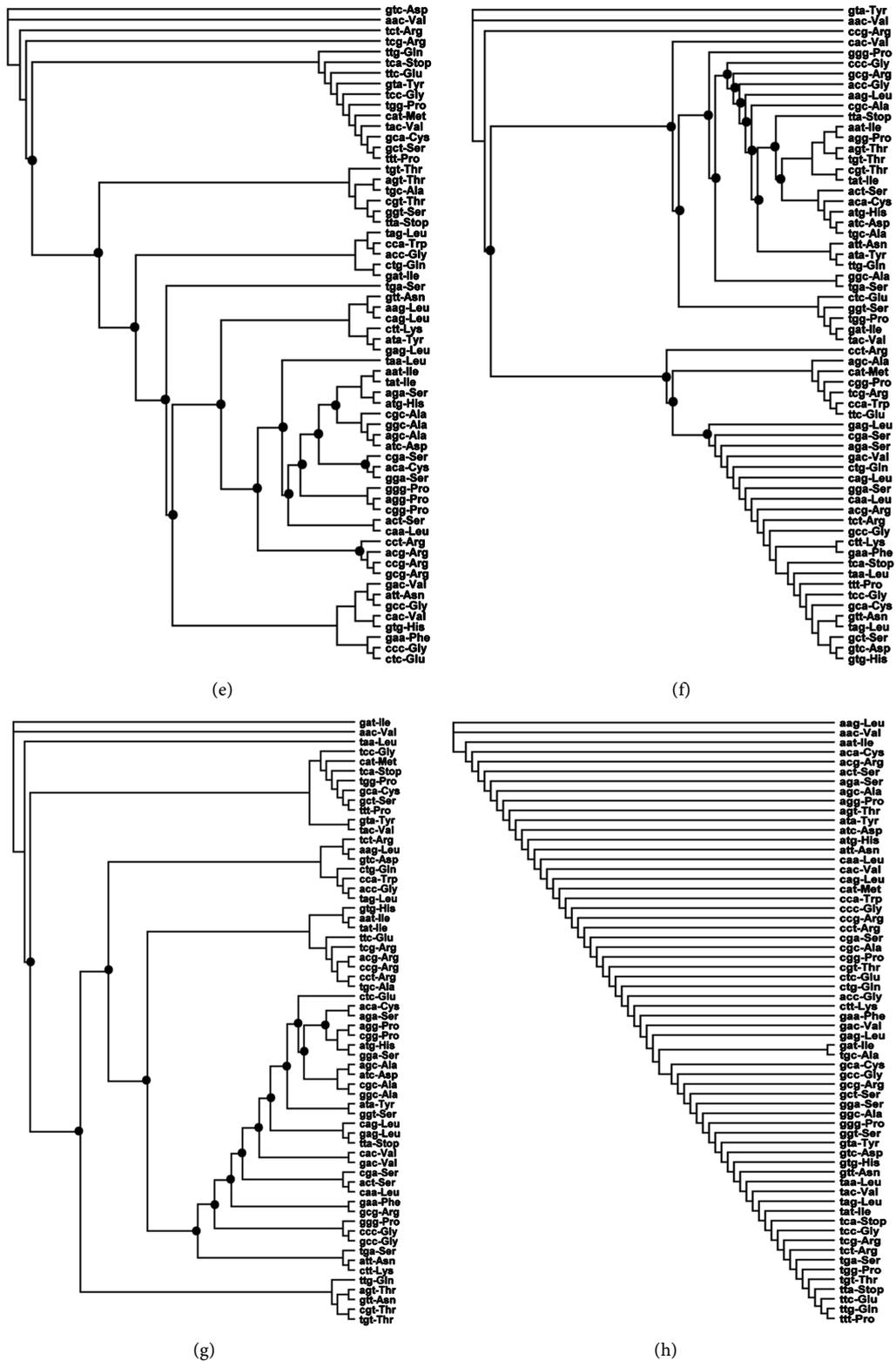


Figure 2. Phylogenetic trees of 62 cod on sequences with a degree of similarity of $s = 10$, $s = 35$, $s = 45$ and $s = 50$ for the antiparallel phylogenetic tree and antiparallel phylogenetic tree. Each tree branch represents one group codon and its abbreviated letters. The black solid dots (•) in the trees represent the codons that agree with the point mutation mechanism in a branch. (a) Parallel phylogenetic tree with $s = 10$; (b) Antiparallel phylogenetic tree with $s = 10$; (c) Parallel phylogenetic tree with $s = 35$; (d) Antiparallel phylogenetic tree with $s = 35$; (e) Parallel phylogenetic tree with $s = 45$; (f) Antiparallel phylogenetic tree with $s = 45$; (g) Parallel phylogenetic tree with $s = 50$; (h) Antiparallel phylogenetic tree with $s = 50$.

parallel phylogenetic tree with $s = 35$ was more compact compared with the parallel phylogenetic tree with $s = 35$, *i.e.*, three small clusters, one large cluster, and two isolated branches comprised the entire tree. Two clusters formed the core of the larger cluster. However, it appeared to be more dispersed than the antiparallel phylogenetic tree with $s = 10$. Surprisingly, most of the codons in the clusters did not have template duplication codons, even though they had copies upstream. Their evolution did not agree with the “template duplication mechanism.”

As the degree of similarity (s) increased, more dispersed clusters appeared in the larger cluster in the parallel phylogenetic tree. As shown in **Figure 2(e)**, the largest cluster is divided into six small clusters with $s = 45$, where gtc, aac, tct, and tcg remained in isolated branches. A comparison of **Figure 2(a)**, **Figure 2(c)**, and **Figure 2(e)** shows that the codon aac occupies a basal clade and it did not vary with the degree of similarity (s) in the three phylogenetic trees. The evolutionary relationships among the branches of the three phylogenetic trees were always ordered and stable with different values of s . Most tRNA anticodons in the same clusters had a high match with the “point mutation mechanism” and most had only one base difference from the anticodon sites, which suggests that they evolved by mutating from the same ancestral sequences via point mutations. At the same time, many isoaccepting tRNA codons were distributed in the same cluster and they had a very compact structure. However, not all of the isoaccepting tRNA codons had neighboring relationships, *e.g.*, four codons in the isoaccepting group for *Arg* were neighboring whereas the other two were far from their branch, which suggested that these two codons may have evolved from neighboring codons. In contrast to the sparse distribution of the parallel phylogenetic tree, the antiparallel phylogenetic tree with $s = 45$ comprised two large clusters with a more compact branch distribution. One large cluster also appeared to have a ladder-like rank. Similar results are shown in **Figure 2(f)** where more isoaccepting tRNA codons had neighbors in the same cluster, *e.g.*, five codons for *Leu* were adjacent in a large cluster. However, not all of the isoaccepting tRNA codons were allocated to the same cluster. **Figure 2(f)** shows that evidence for duplication was absent from the entire tree.

As the degree of similarity s increased to 50, the distances of most codon branches in the parallel and antiparallel phylogenetic trees tended toward 1, as shown in **Figure 2(g)**. The parallel phylogenetic trees with $s = 50$ displayed a ladder-like rank, but there were few isolated branches and the distances among most branches were similar. There were also many small clusters that contained some codons of isoaccepting tRNAs. Most of these codons showed no evidence of point mutations at higher levels in the upstream branch. With $s > 50$, most of the distances for the codons in the parallel phylogenetic trees were equal to 1. There were clear differences in the parallel phylogenetic trees in **Figure 2(h)**, while most of the distances among codon branches in the antiparallel phylogenetic trees with $s = 50$ were equal to 1, and the distribution of the tree was a ladder-like rank.

4. Conclusions

We constructed $62 \times 61/2$ groups of networks and eight phylogenetic trees of tRNAs based on a point mutation mechanism and a complete duplication mechanism. We analyzed the features of each codon branch and the phylogenetic trees in parallel and antiparallel directions. We found that point mutations had an important role in the evolution of the 62 codon sequence groups. Point mutations were found in the codon sites in the same clusters in four complete parallel phylogenetic trees, whereas there was no evidence for a complementary duplication mechanism in the corresponding antiparallel phylogenetic trees. In addition, many codons of isoaccepting tRNA were found in neighboring clusters or very close clusters. The codons with one site difference were very close together. These results support the hypothesis that new tRNA genes were recruited from one isoacceptor group to another via a point mutation in the sequence.

Surprisingly, most of the codons in the antiparallel phylogenetic trees in the same cluster did not have complementary copies without the point mutation codons, although they were compared in the antiparallel direction. As shown in **Figure 3**, most codons in the antiparallel phylogenetic tree with $s = 45$ had no

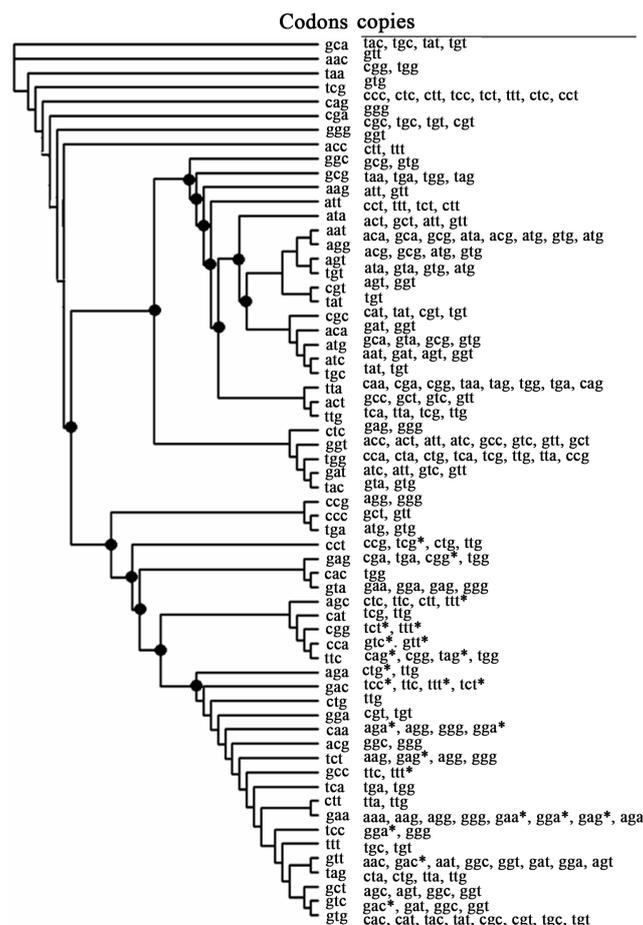


Figure 3. Codons in the antiparallel phylogenetic tree where $s = 45$ and its possible complementary duplication anticodons. The symbol “*” indicates that this anticodon has a copy in the cluster.

complementary codons, although many had point mutation codons in the same cluster. These observations also applied to the other antiparallel trees with different s values. They are marked with a black solid dot (●) in all of the trees and they provide powerful support for the role of point mutations in the evolution of the 64 tRNA codon sequences.

Point mutations occur in the mitochondria tRNA; some positions will cause loss of stability or produce disease. As more and more tRNA sequencing, the mutation of the tRNA gene sequences is studied further, and the evolutionary mechanisms of the RNA will be proven.

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