

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

**LS^x: Automated reduction of gene-specific lineage
evolutionary rate heterogeneity for multi-gene phylogeny
inference**

Carlos J. Rivera-Rivera^{1,2} and Juan I. Montoya-Burgos^{1*}

¹ Department of Genetics and Evolution, University of Geneva, Switzerland

² Institute of genetics and genomics in Geneva (iGE3), University of Geneva, Switzerland

* Corresponding author: juan.montoya@unige.ch

ABSTRACT

26

27 **Motivation:** LS³ is a recently published algorithm to reduce lineage evolutionary rate heterogeneity,
28 a condition that can produce inference artifacts in molecular phylogenetics. The LS³ scripts are
29 Linux-specific and the criterion to reduce lineage rate heterogeneity can be too stringent in datasets
30 with both very long and very short branches.

31

32 **Results:** LS^x is a multi-platform user-friendly R script that performs the LS³ algorithm, and has
33 added features in order to make better lineage rate calculations. In addition, we developed and
34 implemented an alternative version of the algorithm, LS⁴, which reduces lineage rate heterogeneity
35 not only by detecting branches that are too long but also branches that are too short, resulting in less
36 stringent data filtering.

37

38 **Availability:** The LS^x script LSx_v.1.1.R and the user manual are available for download at:
39 <https://genev.unige.ch/research/laboratory/Juan-Montoya>

INTRODUCTION

40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

We recently showed that biases emerging from evolutionary rate heterogeneity among lineages in multi-gene phylogenies can be reduced with a sequence data subselection algorithm to the point of uncovering the true phylogenetic signal (Rivera-Rivera and Montoya-Burgos 2016).

In that study, we presented an algorithm called Locus Specific Sequence Subsampling (LS³), which reduces lineage evolutionary rate heterogeneity gene-by-gene in multi-gene datasets. For each gene alignment, LS³ implements a likelihood ratio test (LRT) (Felsenstein 1981) between two models. One model assumes equal rates of evolution among all ingroup lineages (single rate model) and the other model assumes that three (or more), user-defined ingroup lineages have their own independent rate of evolution (multiple rates model). If the multiple rates model fits the data significantly better than the single rate model, the branch lengths of the phylogeny are estimated, the fastest-evolving sequence is removed, and the new reduced gene sequence dataset is tested again with the LRT. This process is iterated until a set of ingroup taxa is found whose lineage evolutionary rates can be explained equally well by the single rate model or the multiple rates model. The fast-evolving sequences that had to be removed from each gene alignment to reach this point are flagged as potentially problematic due to their higher evolutionary rate, and gene datasets which never reached this point are flagged as a whole as potentially problematic (Rivera-Rivera and Montoya-Burgos 2016). LS³ proved to be effective in reducing long branch attraction (LBA) artifacts in simulated nucleotide data and in two biological multi-gene datasets (one in nucleotides, and one in amino acids), and its potential to reduce phylogenetic biases has been recognized by several other authors (Cruaud and Rasplus 2016; Suh 2016; Bleidorn 2017).

Concomitantly to the publication of the LS³ algorithm, a set of bash scripts were made available to perform these tasks automatically (<http://genev.unige.ch/en/users/Juan-Montoya/unit>). While these bash scripts are effective, they only run in Linux systems, and the layout and programming is not entirely user-friendly. This prompted us to produce a new, re-programmed version of the LS³ algorithm which is user-friendly, contains important new features, and can be

66 used across platforms. For this new implementation we also developed a second data subselection
67 algorithm based on LS³, called “LS³ supplement” (LS⁴) which subselects sequences for lineage
68 evolutionary rate homogeneity by not only removing very long branches, but also very short ones.

69

70

LS^X DESCRIPTION

71 The new script is called LS^X, is entirely written in R (R Core Team 2016), and uses PAML
72 (Yang 2007) and the R packages *ape* (Paradis et al. 2004; Popescu et al. 2012) and *adephylo*
73 (Jombart et al. 2010). If PAML, R, the R packages and Rscript are installed and functional, the
74 script runs regardless of the platform, with all parameters given in a single raw text control file. As
75 with the bash implementation of LS³, LS^X reads alignments in PHYLIP format, but unlike the
76 former, it produces for each gene a version of the alignment including only the sequences that
77 satisfied the lineage rate homogeneity criterion. In LS^X, the best model of sequence evolution can be
78 given for each gene (in the original implementation only a single model could be selected for all
79 genes), thus improving the accuracy of the branch length estimations and likelihood calculations
80 under PAML. In addition, the users can select more than three lineages of interest for the lineage
81 evolutionary rate heterogeneity test (Fig. 1a,b), while the bash implementation was hardcoded to
82 work with only three lineages of interest.

83

84

LS³: AN LS⁴ SUPPLEMENT

85 Within LS^X we also implemented LS⁴, a second data subselection algorithm that is
86 optimized for datasets in which not only extremely long branches but also extremely short branches
87 are present in the starting phylogenetic tree. While both LS³ and LS⁴ follow the same general
88 algorithm, they differ in the criterion for choosing the sequence to be removed in the sequence
89 subselection steps. Under LS³, the fastest-evolving of the ingroup sequences is removed in each
90 iteration, as determined by its calculated sum of branch length (SBL), starting from the stem of the
91 ingroup (Fig. 2a). This can lead to the flagging of too much data in a dataset containing extremely

92 slow-evolving sequences (Fig. 2*b*). In such cases, under LS³, not only the fast-evolving sequences
 93 will be removed, but also the sequences with intermediate evolutionary rates which are still
 94 evolving “too fast” relative to the extremely slow-evolving ones (Fig. 2*b*). While this approach is
 95 not incorrect, simply very stringent, it can lead to the flagging of too many sequences in certain
 96 datasets and to poorly-resolved phylogenies when concatenating and analyzing the remaining data.

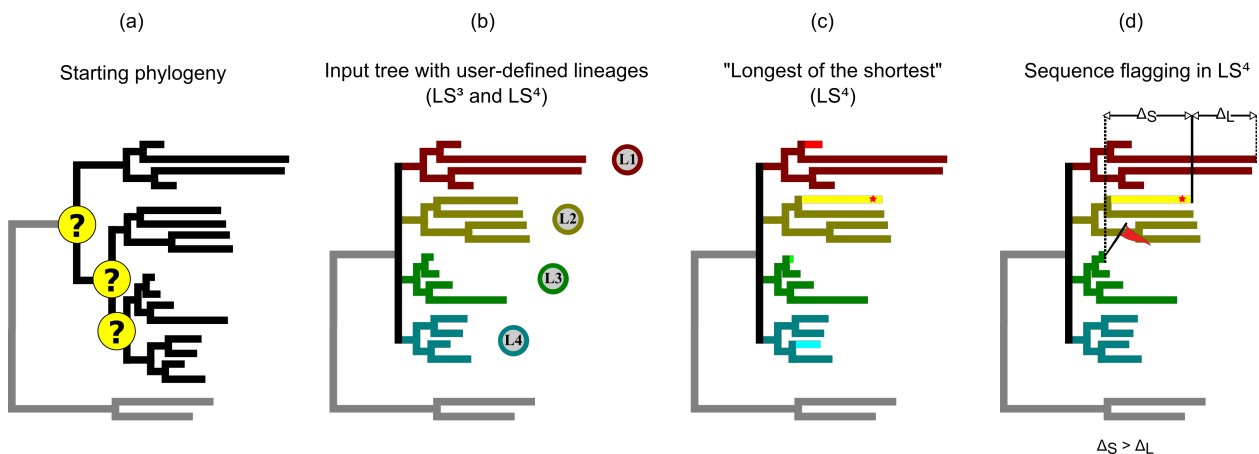


Figure 1. Schematic representation of the procedure for flagging sequences in LS⁴. In (a), the general phylogeny for this group of taxa, with the nodes in question highlighted. For for LS³ and LS⁴, an input tree is given in which the nodes into question are collapsed, and the lineages involved are identified (b). In (c), LS⁴ identifies the shortest branch for each lineage (highlighted), and then identifies the longest among them (red star). The sequence to be removed in each iteration of LS⁴ is the one with the tip furthest from the tip of the “longest of the shortest” branch (d), resulting in the flagging of both extremely long and extremely short branches.

98 In order to allow for the inclusion of more data while still reaching lineage rate
 99 homogeneity, LS⁴ employs a different criterion which considers both too fast- and too slow-
 100 evolving sequences for removal. Under LS⁴, when the SBLs for all ingroup sequences of a given
 101 gene are calculated, they are grouped by the user-defined lineage of interest to which they belong.
 102 The shortest branch of each lineage of interest is identified, and then the longest among them across
 103 all ingroup lineages (“the longest of the shortest”, see Fig. 1c) is picked as a benchmark. Because in
 104 both LS³ and LS⁴ each lineage of interest has to be represented by a minimum of species (defined
 105 by the user), this “longest of the shortest” branch represents the slowest evolutionary rate at which
 106 all lineages could converge. Then, in the sequence subselection steps, the sequence to be removed is
 107 the ingroup sequence for which the absolute value of the difference between its SBL and the SBL of
 108 the benchmark sequence is the largest. In other words, for a given gene, the sequence removed in
 109 each iteration of LS⁴ is that which produces the tip furthest from the benchmark, be it faster- or

110 slower-evolving (Fig. 1d). We are confident that the criterion used in LS⁴ will result in less genes
111 flagged completely (as compared to LS³) when extremely slow-evolving sequences are present (Fig.
112 2b).

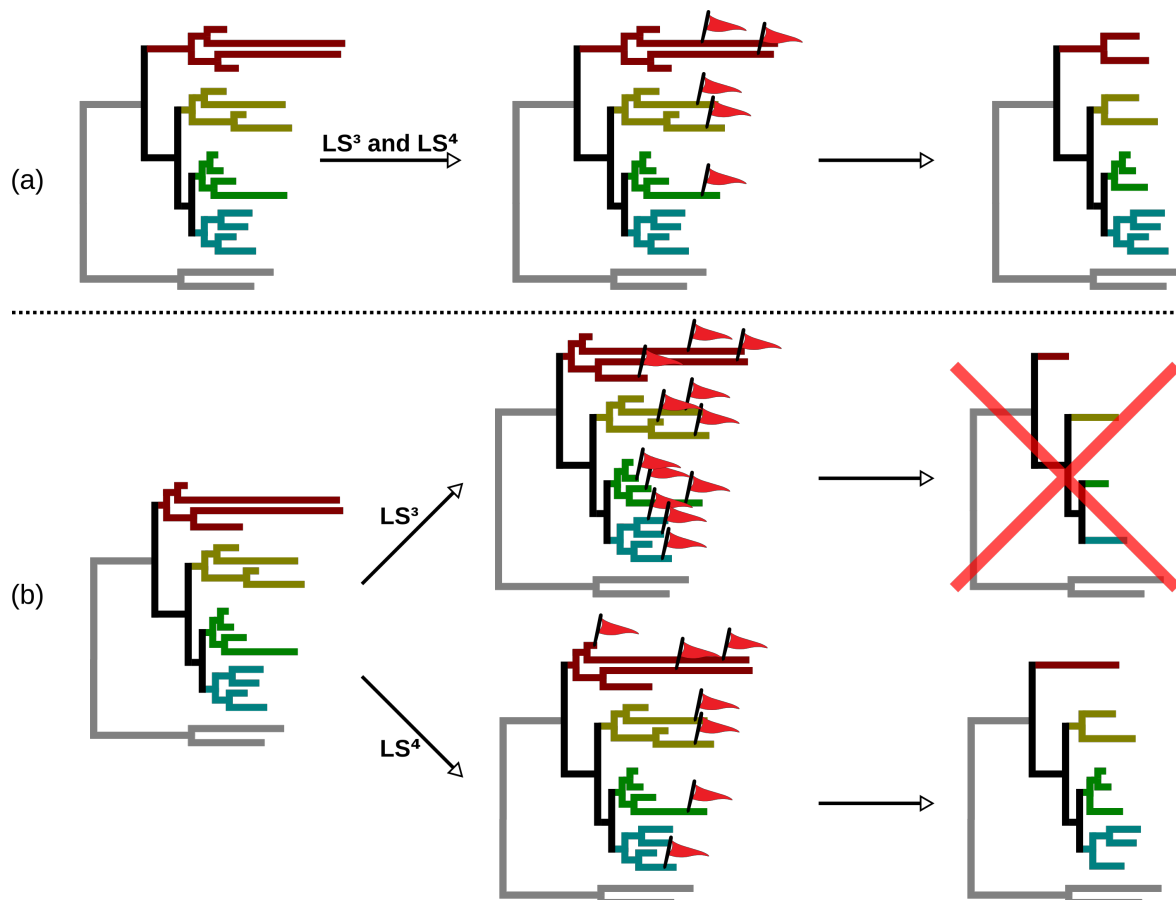


Figure 2. A schematic representation of the different ways that LS³ and LS⁴ reach lineage rate homogeneity for a given gene sequence dataset. In (a), a dataset with a general rate homogeneity with the exception of several faster evolving branches. In this case, both methods will flag the faster evolving sequences, and reach the same taxon subset with homogeneous rates of evolution. In (b), a dataset with general lineage rate heterogeneity, and with a very short branch (the top branch of that tree). In such a case, LS³ will remove all of the faster evolving sequences, until only the slowest sequence of each clade of interest remains. At this point, that gene sequence dataset is flagged completely as problematic because lineage rate heterogeneity is still too strong. In contrast, LS⁴ will remove the faster evolving sequences and also the slowest one, thus reaching lineage rate homogeneity for this dataset.

113

ACKNOWLEDGEMENTS

114 We are deeply thankful to Jose Nunes for his help and suggestions during the programming
115 of LS^x in R, and Joe Felsenstein for discussions about the subselection criterion used in the LS⁴
116 algorithm. This research was funded by the Swiss National Science Foundation (grant
117 31003A_141233 to JIMB) and the Institute for Genetics and Genomics in Geneva (iGE3).

118

119

REFERENCES

- 120 Bleidorn C. 2017. *Phylogenomics: An Introduction*. page 9. Springer International Publishing AG
- 121 Cruaud A, Rasplus JY. 2016. Testing cospeciation through large-scale cophylogenetic studies. *Curr.*
122 *Opin. Insect Sci.* 18:53–59.
- 123 Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J.*
124 *Mol. Evol.* 17:368–376.
- 125 Jombart T, Balloux F, Dray S. 2010. Adephylo: New Tools for Investigating the Phylogenetic Signal
126 in Biological Traits. *Bioinformatics* 26:1907–1909.
- 127 Paradis E, Claude J, Strimmer K. 2004. APE: Analyses of Phylogenetics and Evolution in R
128 language. *Bioinformatics* 20:289–290.
- 129 Popescu AA, Huber KT, Paradis E. 2012. Ape 3.0: New tools for distance-based phylogenetics and
130 evolutionary analysis in R. *Bioinformatics* 28:1536–1537.
- 131 Rivera-Rivera CJ, Montoya-Burgos JI. 2016. LS³: A Method for Improving Phylogenomic
132 Inferences When Evolutionary Rates Are Heterogeneous among Taxa. *Mol. Biol. Evol.*
133 33:1625–1634.
- 134 Suh A. 2016. The phylogenomic forest of bird trees contains a hard polytomy at the root of
135 *Neoaves*. *Zool. Scr.* 45:50–62.
- 136 R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for
137 Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- 138 Yang Z. 2007. PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* 24:1586–
139 1591.