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A simple method for data partitioning based on relative evolutionary rates

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Background. Multiple studies have demonstrated that partitioning of molecular datasets is important in model-based phylogenetic analyses. Commonly, partitioning is done *a priori* based on some known properties of sequence evolution, e.g. differences in rate of evolution among codon positions of a protein-coding gene. Here we propose a new method for data partitioning based on relative evolutionary rates of the sites in the alignment of the dataset being analysed. The rates are inferred using the previously published Tree Independent Generation of Evolutionary Rates (TIGER), and the partitioning is conducted using our novel python script RatePartitions. We applied this method to eight published multi-locus phylogenetic datasets, representing different taxonomic ranks within the insect order Lepidoptera (butterflies and moths).

Methods. We used TIGER to generate relative evolutionary rates for all sites in the alignments. Then, using RatePartitions, we partitioned the data into bins based on their relative evolutionary rate. RatePartitions applies a simple formula that ensures a distribution of sites into partitions following the distribution of rates of the characters from the full dataset. This ensures that the invariable sites are placed in a partition with slowly evolving sites, avoiding the pitfalls of previously used methods, such as *k*-means. Different partitioning strategies were evaluated using BIC scores as calculated by PartitionFinder.

Results. In all eight datasets, partitioning using TIGER and RatePartitions was significantly better as measured by the BIC scores than other partitioning strategies, such as the commonly used partitioning by gene and codon position.

Discussion. We developed a new method of partitioning phylogenetic datasets without using any prior knowledge (e.g. DNA sequence evolution). This method is entirely based on the properties of the data being analysed and can be applied to DNA sequences (protein-coding, introns, ultra-conserved elements), protein sequences, as well as morphological characters. A likely explanation for why our method performs better than other tested partitioning strategies is that it accounts for the heterogeneity in the data to a much greater extent than when data are simply subdivided based on prior knowledge.

1 **A simple method for data partitioning based on relative evolutionary rates**

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7 **Abstract**

8 **Background.** Multiple studies have demonstrated that partitioning of molecular datasets is
9 important in model-based phylogenetic analyses. Commonly, partitioning is done *a priori* based
10 on some known properties of sequence evolution, e.g. differences in rate of evolution among
11 codon positions of a protein-coding gene. Here we propose a new method for data partitioning
12 based on relative evolutionary rates of the sites in the alignment of the dataset being analysed.
13 The rates are inferred using the previously published Tree Independent Generation of
14 Evolutionary Rates (TIGER), and the partitioning is conducted using our novel python script
15 RatePartitions. We applied this method to eight published multi-locus phylogenetic datasets,
16 representing different taxonomic ranks within the insect order Lepidoptera (butterflies and
17 moths).

18 **Methods.** We used TIGER to generate relative evolutionary rates for all sites in the alignments.
19 Then, using RatePartitions, we partitioned the data into bins based on their relative evolutionary
20 rate. RatePartitions applies a simple formula that ensures a distribution of sites into partitions
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22 invariable sites are placed in a partition with slowly evolving sites, avoiding the pitfalls of
23 previously used methods, such as *k*-means. Different partitioning strategies were evaluated using
24 BIC scores as calculated by PartitionFinder.

25 **Results.** In all eight datasets, partitioning using TIGER and RatePartitions was significantly
26 better as measured by the BIC scores than other partitioning strategies, such as the commonly
27 used partitioning by gene and codon position.

28 **Discussion.** We developed a new method of partitioning phylogenetic datasets without using any
29 prior knowledge (e.g. DNA sequence evolution). This method is entirely based on the properties
30 of the data being analysed and can be applied to DNA sequences (protein-coding, introns, ultra-
31 conserved elements), protein sequences, as well as morphological characters. A likely explanation

32 for why our method performs better than other tested partitioning strategies is that it accounts for
33 the heterogeneity in the data to a much greater extent than when data are simply subdivided based
34 on prior knowledge.

35 **Key words:** BIC; intron; PartitionFinder; phylogenetics; phylogenomics; RatePartitions; UCEs;
36 TIGER

37 Introduction

38 Phylogenetic analysis of DNA sequences is based on models of molecular evolution that estimate
39 parameters such as base frequencies, substitution rates among nucleotides, as well as among-site
40 rate variation. To reduce the heterogeneity in the data, datasets are often partitioned into subsets
41 that are deemed to have undergone more similar molecular evolution. A number of studies have
42 demonstrated that partitioning of data is important (Nylander et al., 2004; Brandley, Schmitz &
43 Reeder, 2005; Brown & Lemmon, 2007; Rota, 2011; Rota & Wahlberg, 2012; Kainer & Lanfear,
44 2015), especially for model-based phylogenetic analyses, which are known to be more sensitive
45 to underparameterization than overparameterization (Huelsenbeck & Rannala, 2004; Lemmon &
46 Moriarty, 2004; Nylander et al., 2004).

47 Today, in most phylogenetic studies, partitions are defined *a priori* by the user, commonly
48 by gene, gene and codon position, stems vs. loops in ribosomal RNA, or another feature of the
49 sequence that the user believes to be important. In several studies, partitioning of protein-coding
50 genes by gene and codon position was demonstrated to be a better option when compared to not
51 partitioning or partitioning by gene (Nylander et al., 2004; Brandley, Schmitz & Reeder, 2005;
52 Brown & Lemmon, 2007; Miller, Bergsten & Whiting, 2009; Rota, 2011). This approach is
53 practical when a dataset consists of only a few genes. However, when data come from tens (or
54 hundreds) of genes, this approach becomes unwieldy, although there are methods that allow one
55 to combine many *a priori* established partitions into fewer, based on model testing with programs
56 such as PartitionFinder (Lanfear et al., 2012).

57 Using a method described by Cummins and McInerney (2011), it is possible to partition a
58 dataset in a more objective way, based on the properties of the data. The method takes into
59 account the relative evolutionary rates of characters by comparing the patterns in character-state
60 distributions in homologous characters (i.e., nucleotides or amino acids in a molecular alignment
61 or characters in a morphological matrix). Each character thus receives a value for its evolutionary

62 rate, which is based on comparisons to all other characters in the matrix. The rate values can then
63 be used to group characters with similar rates by dividing the range of rates into bins, which can
64 be user-defined so as to span equal ranges of rates. This usually leads to the first bin containing
65 characters that are invariable, and the last bin consisting of characters with the highest relative
66 rate of change (Cummins & McInerney, 2011). This method is implemented in the program
67 TIGER – Tree Independent Generation of Evolutionary Rates (Cummins & McInerney, 2011).

68 Originally, the method was developed to identify and exclude the fastest-evolving
69 characters in a dataset, but this approach has potential problems (see Simmons & Gatesy, 2016).
70 We have extended the TIGER method to partitioning the data by sorting characters into data
71 subsets with similar relative rates of evolution (Rota & Wahlberg, 2012; Rota & Miller, 2013;
72 Wahlberg et al., 2014), where we arbitrarily combined neighbouring TIGER bins to form data
73 partitions with enough characters for analysis. A similar approach has been used in a number of
74 studies (Kaila et al., 2013; Rota & Miller, 2013; Heikkilä et al., 2014; Matos-Maravi et al., 2014;
75 Wahlberg et al., 2014; Edger et al., 2015; Kristensen et al., 2015; Rajaei et al., 2015; Ounap,
76 Viidalepp & Truuverk, 2016), and although this method works quite well, the downside is that it
77 requires the user to make a subjective decision about the final partitioning strategy.

78 Recently, a different way of using TIGER together with *k*-means was described by
79 Frandsen et al. (2015). They compared their new method to traditional *a priori* defined partitions,
80 as well as to site rates calculated using a maximum likelihood function. In all test cases,
81 partitioning by both TIGER calculated rates and likelihood calculated rates performed better than
82 traditional methods, with likelihood rates doing much better (Frandsen et al., 2015). However, the
83 *k*-means algorithm has been found to place all invariable characters into one partition (Baca et al.,
84 2017), which leads to biased likelihood values. Indeed, the *k*-means algorithm has now been
85 disabled for molecular data in PartitionFinder2

86 (<https://github.com/brettc/partitionfinder/commit/19d7fe41d2e469c131a5b0cc30184a069867b7f2>
87 accessed 13 November 2017).

88 Here, we describe a simple and objective method for partitioning using TIGER. TIGER is
89 again used for sorting of sites based on their relative evolutionary rates, but now we introduce an
90 algorithm – RatePartitions – for dividing the sites among partitions in an objective way. This
91 method has already been used in several published studies (Heikkilä et al., 2015; Rota, Pena &
92 Miller, 2016; Rota et al., 2016; Sahoo et al., 2016). We report our findings from further testing
93 RatePartitions performance on eight published datasets, some of which were difficult to analyse
94 using traditional partitioning strategies. We use the Bayesian Information Criterion (BIC) for
95 comparison of partitioning strategies. We do not carry out phylogenetic analyses and compare
96 resulting topologies because it has been previously established that partitioning does affect
97 topology, branch support, and branch lengths (see Kainer & Lanfear, 2015 and references
98 therein), and since true phylogenies in all of these cases are unknown, we can only select the best
99 partitioning strategy using statistical model evaluation metrics, such as e.g. BIC.

100 **Materials & Methods**

101 *RatePartitions*

102 Although it is technically incorrect to use the word ‘partition’ when referring to a data subset, we
103 use ‘partition’ in that sense since this is commonly done in phylogenetics. When partitioning is
104 carried out using TIGER, one must take into account the general properties of the data. One of
105 these properties is that with standard DNA sequence data of protein-coding genes, one to two
106 thirds of the data consist of invariable characters. These tend to be binned together to the
107 exclusion of other data when using the TIGER binning strategy or the k -means algorithm (Baca et
108 al., 2017). A partition made of only such data contains no phylogenetic information and thus it is
109 advisable to include a number of slowly evolving characters to create a data partition with low

110 variation. To deal with that problem we developed RatePartitions – an algorithm which works in
111 the following way. The dataset is first run in TIGER to calculate the relative rate of evolution for
112 each site (character). These values can range from 1 (invariable sites) to 0 (no common patterns,
113 i.e. the fastest-evolving sites). The sites are then combined into partitions using RatePartitions,
114 which applies a simple formula that ensures a distribution of sites into partitions following the
115 distribution of rates of the characters from the full dataset. This leads to larger partitions for
116 characters with slower rates and, conversely, smaller partitions for those with higher rates.
117 Preliminary tests using MrModeltest v2.3 (Nylander, 2004) and PartitionFinder v.1.0.0 (Lanfear
118 et al., 2012) suggested that this strategy led to models with uniform rate variation within
119 partitions.

120 RatePartitions is a PYTHON script (Supplemental Script S1) that determines the rate-
121 spans for a variable number of partitions based on a user-specified division factor and the original
122 range of rates calculated by TIGER (with the “-rl” command), and subsequently defines character
123 sets for each partition. The rate-spans are calculated for the first (and slowest) partition with the
124 following function:

$$125 \quad z = x - ((x - y) / d)$$

126 and for the remaining partitions:

$$127 \quad z = x - ((x - y) / (d + p * 0.3))$$

128 where z is the lower limit of the rate-span, x is the upper limit of the rate-span (determined
129 iteratively for each partition, i.e. z becomes x in the following iteration), y is the minimum value
130 of rates for the entire dataset, d is a user defined division factor (which must be greater than 1; a
131 higher number gives a greater number of partitions) and p is the partition number (when >1),
132 which is multiplied by a fixed value of 0.3. The latter reduces the rate-span exponentially as
133 partition number grows, which we found leads to partitions with more uniform rate variation for
134 model-based analyses. Thus, for a dataset with rates ranging from 1 to 0.2 and with d set to 1.5,

135 the first partition will consist of all characters with rates between 1 and $1 - ((1 - 0.2) / 1.5) = 0.4667$.
136 For partition 2, $x = 0.4667$ and this partition will include characters with rates between 0.4667
137 and $0.4667 - ((0.4667 - 0.2) / (1.5 + 2 * 0.3)) = 0.3397$, and so on until less than 10% of all characters
138 are remaining. At this point the iterations are stopped and the remaining characters are placed into
139 their own partition (which becomes the last and fastest-evolving partition).

140 *Data partitioning and analyses*

141 We analysed eight previously published lepidopteran datasets (Kodandaramaiah et al., 2010;
142 Sihvonen et al., 2011; Penz, Devries & Wahlberg, 2012; Rota & Wahlberg, 2012; Zahiri et al.,
143 2013; Matos-Maravi et al., 2014; Wahlberg et al., 2014; Rönkä et al., 2016) (Table 1). From the
144 published datasets we excluded sites from the alignment that had more than 80% of missing data
145 unless they had 1% or fewer of such sites (Table 2). These were the following datasets: Arctiina,
146 Geometridae, *Morpho*, and Pieridae. All datasets are provided as Supplemental Information (Data
147 S1). The datasets varied in base pair length from 4435 to 6372 and in number of taxa from 31 to
148 164 (Table 1). All datasets included one mitochondrial gene (COI) and four to seven nuclear
149 genes that are commonly used in lepidopteran phylogenetics (CAD, EF-1 α , GAPDH, IDH,
150 MDH, RpS5, wingless) (Wahlberg & Wheat, 2008). We compared 14 partitioning strategies
151 (Table 2), including user-defined ones such as partitioning by gene and by gene and codon
152 position, and a number of different strategies devised based on the relative evolutionary rates
153 assigned by TIGER and division of sites into partitions using the RatePartitions algorithm. We
154 varied the parameter d in the RatePartitions algorithm between 1.5 and 4.5 in increments of 0.5.
155 For comparison of the partitioning strategies we used the BIC score as calculated by
156 PartitionFinder 1.1 (Lanfear et al., 2012). We did two types of searches with PartitionFinder. The
157 first was a user-defined search for direct evaluation of the partitioning strategy obtained with

158 TIGER and RatePartitions. The second was a greedy search, which searches for partitions with
159 similar parameter estimates and combines them so as to reduce the final number of partitions. For
160 example, for a dataset with eight genes that are *a priori* partitioned by gene and codon position
161 (24 partitions), a greedy search may result in a total of nine partitions because some of the
162 original partitions were combined into a larger subset of data with similar parameter values. BIC
163 was chosen as a statistical model evaluation metric because it has been shown to perform well in
164 model selection for phylogenetic analysis (Abdo et al., 2005). We refer to analyses with different
165 values of d as TIG1.5, TIG2.0, etc. The greedy search was not performed on TIG1.5, TIG2.0,
166 TIG2.5, and TIG3.0 partitioning strategies because these were shown to have inferior BIC values
167 in preliminary analyses.

168 **Results**

169 The eight datasets analysed covered a range of taxonomic ranks within Lepidoptera, from genus
170 level (*Morpho* and *Calisto*), subtribes (Arctiina and Coenonymphina), two small to medium-sized
171 families (Choreutidae and Pieridae, with about 400 and 1100 species, respectively), to two very
172 large families (Geometridae and Noctuidae, with over 23,000 and 11,000 species, respectively)
173 (van Nieukerken et al., 2011). They varied in sequence length from 4423 to 6716 base pairs
174 (Table 1). The amount of missing data was quite variable. The most complete dataset,
175 Coenonymphina, had more than 90% of sites with less than 20% of missing data, while the least
176 complete dataset, Arctiina, had only 21% of sites with less than 20% of missing data (Table 3).

177 TIGER partitioning resulted in a different number of partitions for each dataset, with
178 Geometridae and Pieridae being split into many more partitions than the other datasets (Table 4).
179 For example, at d equalling 4.5, *Morpho*, the dataset with fewest taxa was split into only seven
180 partitions, Pieridae into 20, Geometridae into 24, while all the other datasets ranged 10–14 in
181 their number of partitions.

182 In all cases partitioning by gene region was clearly the worst way to subdivide the data, as
183 determined by BIC scores, and applying the greedy search made little improvement (Fig. 1, Table
184 S2). In all datasets, partitioning using TIGER and RatePartitions was the best strategy. However,
185 in two datasets (Geometridae and Pieridae), partitioning by gene and codon position with a
186 greedy search came close to the best TIGER strategy, although the BIC scores were still
187 significantly higher for the TIGER strategy (Table S1). In all datasets, the improvement in the
188 BIC score from TIG1.5 to TIG3.0 was quite steep, but further differences between TIG3.5,
189 TIG4.0, and TIG4.5, with and without greedy search were relatively small, although the analyses
190 with the greedy search always received a significantly better BIC score. TIG4.5Gr was the best
191 strategy in *Calisto*, Choreutidae, Noctuidae, and Pieridae, whereas TIG4.0Gr was the best
192 strategy in Arctiina, Coenonymphina, Geometridae, and *Morpho* (Fig. 1, Table S1).

193 An examination of the plots of the relative evolutionary rates estimated by TIGER for
194 each gene fragment and codon position reveal differences among gene fragments, as well as sites
195 belonging to the same codon position in the same gene fragment (Figs 2, S1). As expected, in
196 general, first and second codon positions receive a much higher rate (i.e. implying slower change)
197 than third codon positions, but in some genes there is a large proportion of third codon positions
198 that also receive a rate of one, e.g. in the *Morpho* dataset for CAD, EF-1 α , and RpS5 (Fig. S1).
199 Conversely, there are genes that tend to have some fast-changing first and second codon
200 positions, which then receive a relatively low rate. This is usually the case in COI, the
201 mitochondrial gene, but also in several nuclear genes (wingless in all datasets, but also CAD,
202 MDH, and RpS5 in some of the datasets; Figs 2, S1).

203 Discussion

204 Many studies have shown that partitioning of DNA sequence data for phylogenetic analysis is
205 important because it affects the resulting tree topology, branch support, as well as branch lengths
206 (see Kainer & Lanfear, 2015 and references therein). A common approach is to define partitions *a*
207 *priori* based on some feature(s) of the DNA sequences such as genes, codon positions, stems,
208 loops, introns, exons, etc., but this can be problematic because the properties of the sequence data
209 are not fully known to the user to begin with. To avoid *a priori* partitioning, we developed a
210 method of partitioning based on relative evolutionary rates of sites in an alignment. In our
211 analyses, we demonstrated that this method outperforms other commonly used partitioning
212 strategies, such as partitioning by gene and codon position, in all datasets that we tested. This
213 method is entirely based on the alignment – not on trees or some features of the data deemed
214 important by the user. It can be applied to any kind of categorical data (nucleotides, amino acids,
215 morphological characters), to protein-coding genes, RNA, introns, exons, as well as ultra-
216 conserved elements (UCEs). It can be especially useful for sequences derived from introns or
217 UCEs, where *a priori* partitioning is difficult, as one does not need to provide user-defined
218 partitions.

219 A possible explanation for why TIGER partitioning performed better than partitioning by
220 codon position is that there are significant differences among sites belonging to the same codon
221 position of the same gene in their relative evolutionary rate (Figs 2, S1), and this leads to high
222 heterogeneity in the data when they are simply grouped by codon position. Since our method
223 groups sites based on the pattern present in the alignment, the models of molecular evolution
224 have to account for less variation within each partition.

225 In all of our analyses, partitioning by gene was much worse than the other strategies. A
226 protein-coding gene, with its first, second, and third codon positions, each of which evolve
227 differently, is highly heterogeneous, and applying the same model to such a sequence most likely
228 leads to an underparameterized model. It has been demonstrated that underpartitioning can result

229 in in a more severe error in most datasets than overpartitioning (Brown & Lemmon, 2007; Ward
230 et al., 2010; Kainer & Lanfear, 2015), and our recommendation is to take this into account when
231 devising a partitioning strategy.

232 Our partitioning method has been applied in analyses of several other lepidopteran
233 datasets: 1) the subfamily Acronictinae (Noctuidae) (Rota et al., 2016) analyzed in MrBayes
234 (Ronquist et al., 2012) and RAxML (Stamatakis, 2014); 2) an expanded dataset for the family
235 Choreutidae (Rota, Pena & Miller, 2016) in MrBayes, RAxML, and BEAST (Drummond et al.,
236 2012); 3) the family Hesperiiidae (skippers) (Sahoo et al., 2017) in BEAST; and 4) for inferring
237 relationships among Ditrysian superfamilies and families using molecular and morphological
238 characters (Heikkilä et al., 2015) in RAxML. In the Heikkilä et al. study (2015), in addition to
239 applying our partitioning method, the authors also explored the effect of exclusion of fastest
240 evolving characters from the analyses. They found that phylogenetic signal was lost especially
241 when the fastest evolving morphological characters were excluded, and that branch support was
242 lowered with the exclusion of fastest evolving molecular characters, which also resulted in a
243 spurious placement of some groups, and therefore is not at all recommended (see Simmons &
244 Gatesy, 2016 for a detailed exploration of this topic).

245 An issue we would like to stress with our approach, however, is that it should only be
246 applied to studies where concatenation of data is justified, i.e. where gene tree/species tree
247 problems are minimized. This is because our approach of partitioning by specific properties of
248 each character removes any connections between characters belonging to the same gene region.
249 This reshuffling of characters based on relative rates of evolution does have a biological basis to
250 it (sites evolving at a similar rate are modelled together), but at the risk of losing other
251 biologically relevant information (such as differential evolutionary histories of gene regions). We
252 do feel that for studies looking at deeper relationships, such as among genera, tribes, families, and
253 orders, our approach is very useful and overcomes problems of overpartitioning for large

254 multigene datasets that might be partitioned by codon position, as well as underpartitioning when
255 users might be inclined to analyse their data unpartitioned because they are uncertain of how to
256 partition *a priori*.

257 **Conclusions**

258 Here we present a way of partitioning data based on relative rates of evolution as calculated by
259 TIGER (Cummins & McInerney, 2011). We find that this approach works better than the
260 traditional approaches to partitioning in all of our test cases. Further utility of TIGER calculated
261 rates and RatePartitions needs to be ascertained on other datasets. The program could certainly be
262 used on amino acid (or any other categorical) data in the same way as done here for nucleotides.
263 However, to establish how useful partitioning based on TIGER calculated rates is for
264 phylogenomic data containing sequences from hundreds or thousands of genes, additional testing
265 needs to be conducted.

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268 the Swedish Research Council (NW).

269 **Author contributions**

270 Jadranka Rota conceived and designed the experiments, performed the experiments, analysed the
271 data, wrote the paper, prepared figures and/or tables, and reviewed drafts of the paper.

272 Tobias Malm conceived the RatePartition algorithm, analysed the data and reviewed drafts of the
273 manuscript.

274 Niklas Wahlberg conceived and designed the experiments, performed the experiments, analysed
275 the data, wrote the paper, prepared figures, and reviewed drafts of the paper.

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405 **Tables**

406 **Table 1.** Datasets analysed. List of analysed datasets providing the reference, the number of
407 sampled taxa and gene regions in the dataset, and the length of the dataset in base pairs (bp).

408 **Table 2.** The amount of missing data in each of the eight datasets analysed. All alignment
409 columns were pulled into one of the ten categories based on the range of missing data being 0–
410 10%, 10–20%, etc. to more than 90% missing. The Cumulative missing data refers to summing
411 percentage of missing data from one range category to the next. All datasets had 1% or less of
412 columns in the alignment with missing more than 80% of data, and overall all datasets had 50%
413 or more columns with less than 40% of missing data.

414 **Table 3.** List of partitioning strategies evaluated for each of the analysed datasets. TIGER refers
415 to the program that assigns each site in the alignment a relative evolutionary rate, and d is the
416 division factor in the RatePartitions script used to group sites into subsets based on their relative
417 evolutionary rates. See text for more details.

418 **Table 4.** The number of partitions for each dataset and partitioning strategy. *Gene* refers to
419 partitioning by gene fragment, *Codon* to partitioning by codon position, and *TIG* to partitioning
420 by relative evolutionary rate as estimated with the program TIGER with different values for the d ,
421 division factor in the RatePartitions script. See Table 3 and text for more details.

422 **Figures**

423 **Figure 1.** A comparison of BIC values for the 14 partitioning strategies tested in all eight
424 datasets. The partitioning strategies are plotted on the horizontal axis, and the BIC values are
425 plotted on the vertical axis. The lower the BIC value, the better the partitioning strategy. *Gene*

426 refers to partitioning by gene fragment, *Codon* to partitioning by codon position, and *TIG* to
427 partitioning by relative evolutionary rate as estimated with the program TIGER with different
428 values for the d , division factor in the RatePartitions script. See text and Table 3 for more details.

429 **Figure 2.** Relative evolutionary rate estimates for codon positions in the Noctuidae dataset. Plots
430 are showing the assigned TIGER relative evolutionary rates for codon positions of each of the
431 eight genes in the Noctuidae dataset. TIGER rates are shown on the horizontal axis, and the
432 number of codon positions that were assigned the rate between 0.0–0.1, 0.1–0.2, etc. is shown on
433 the vertical axis. The lower the number, the higher the rate of evolution, with rate of 1 being
434 assigned to invariable sites in the alignment. As expected, most of the first and second codon
435 positions received the rate of 1, but there are exceptions, with some first and/or second codon
436 positions receiving a relatively low rate (especially in e.g. COI, and wgl). Likewise, most of the
437 third codon positions received lower rates, but in some genes (e.g. EF-1 α), the number of third
438 positions that received the TIGER rate of 1 is relatively high. Such plots for the other seven
439 datasets are in supplemental information files Fig. S1.

440 **Supplemental Information**

441 **Supplemental Data File S1.** Datasets analysed in this study. The datasets are provided in the
442 PHYLIP format, together with the RAxML style partition definitions for the best partitioning
443 strategy.

444 **Supplemental Script S1.** RatePartitions script. Python script for grouping sites in the alignment
445 based on the relative evolutionary rate assigned by the program TIGER.

446 **Supplemental Table S1.** Comparison of BIC values. A BIC value is provided for each
447 partitioning strategy for each of the eight datasets analysed.

448 **Supplemental Figure S1.** Relative evolutionary rate estimates for codon positions of all gene
449 fragments. Plots showing the assigned TIGER relative evolutionary rates for codon positions of
450 each of the gene fragments analysed: *Arctiina* (a), *Calisto* (b), *Choreutidae* (c), *Coenonymphina*
451 (d), *Geometridae* (e), *Morpho* (f), and *Pieridae* (g).

Figure 1 (on next page)

A comparison of BIC values for the 14 partitioning strategies tested in all eight datasets

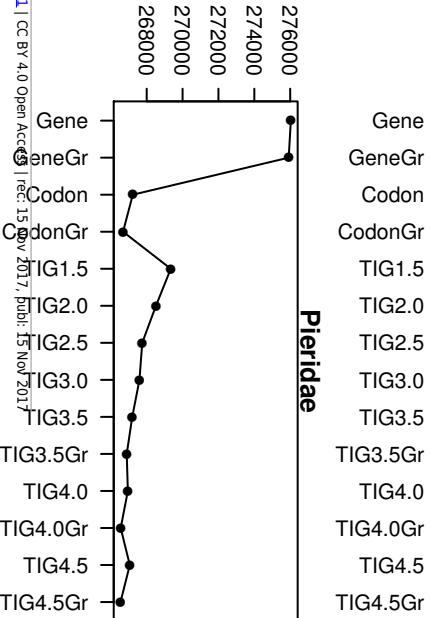
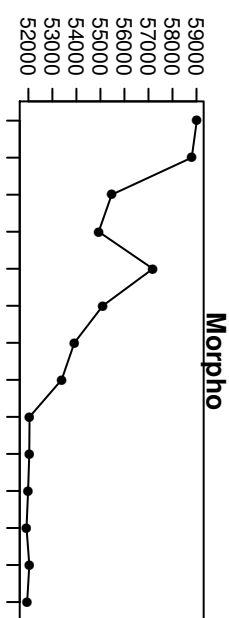
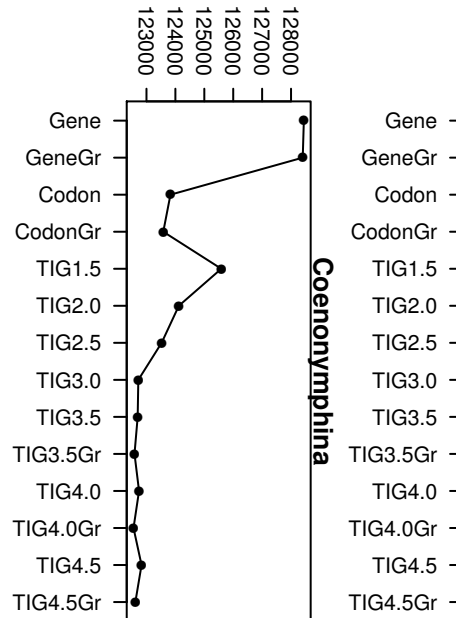
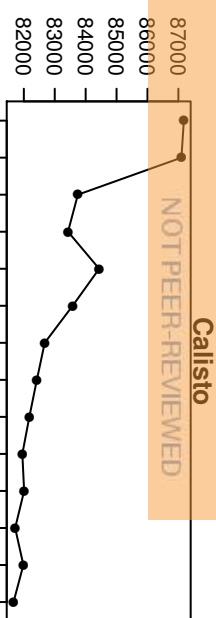
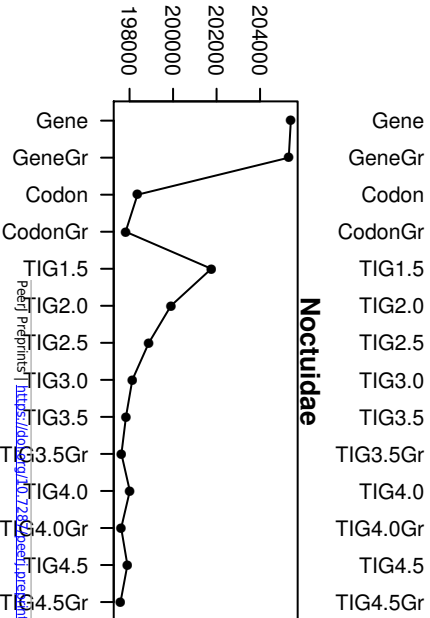
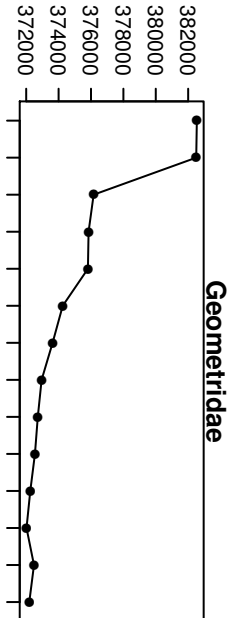
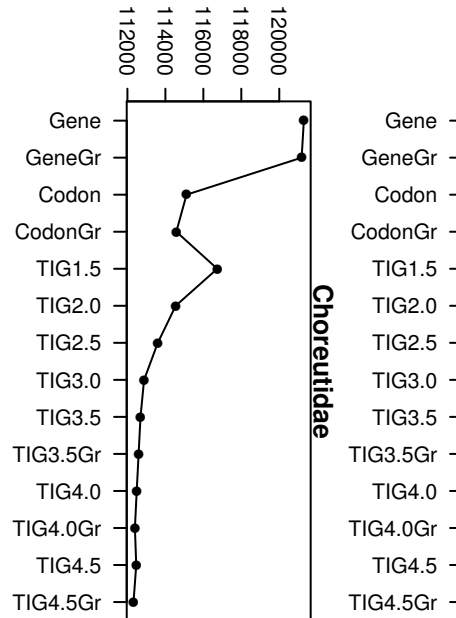
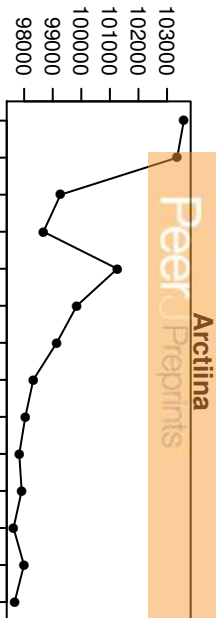
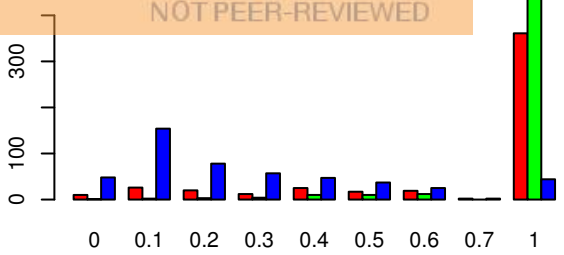
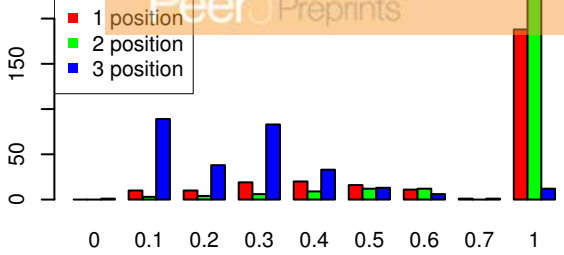
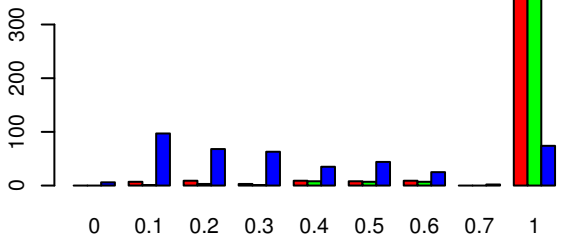


Figure 2 (on next page)

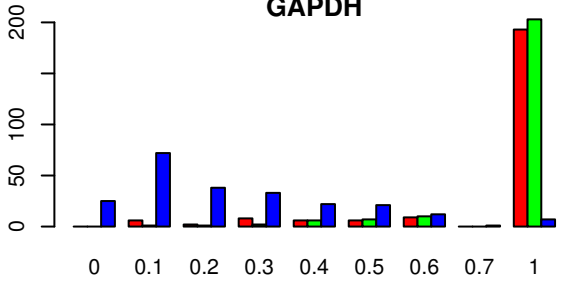
Relative evolutionary rate estimates for codon positions in the Noctuidae dataset



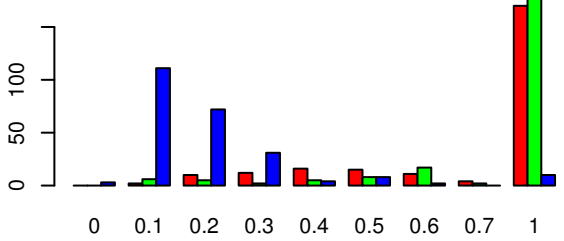
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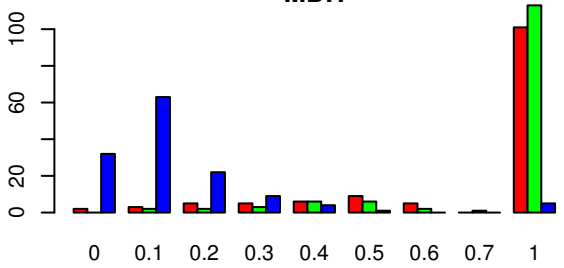
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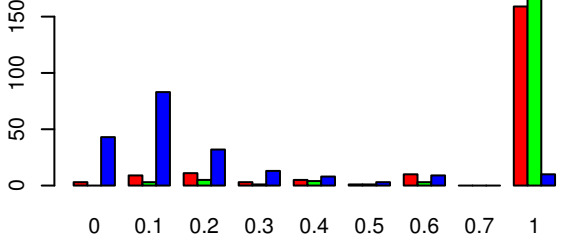
Rates
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Rates
MDH



Rates
RpS5



Rates
wgl

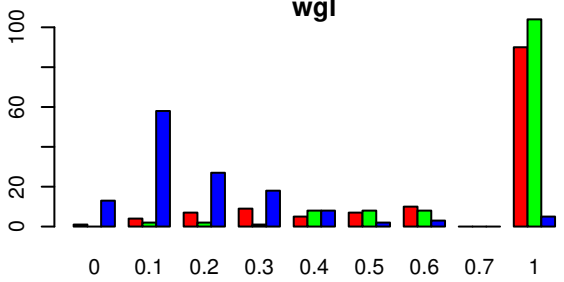


Table 1 (on next page)

Datasets analysed.

List of analysed datasets providing the reference, the number of sampled taxa and gene regions in the dataset, and the length of the dataset in base pairs (bp).

Table 1.

Taxon	Study	No. taxa	No. genes	base pairs
Arctiina	Rönkä et al. 2016	113	8	5809
Calisto	Matos-Maravi et al. 2014	90	6	5297
Choreutidae	Rota & Wahlberg 2012	41	8	6293
Coenonymphina	Kodandaramaiah et al. 2010	69	5	4435
Geometridae	Sihvonen et al. 2011	164	8	5998
Morpho	Penz et al. 2012	31	8	6372
Noctuidae	Zahiri et al. 2013	78	8	6365
Pieridae	Wahlberg et al. 2014	110	8	6247

Table 2 (on next page)

The amount of missing data in each of the eight datasets analysed.

All alignment columns were pulled into one of the ten categories based on the range of missing data being 0-10%, 10-20%, etc. to more than 90% missing. The Cumulative missing data refers to summing percentage of missing data from one range category to the next. All datasets had 1% or less of columns in the alignment with missing more than 80% of data, and overall all datasets had 50% or more columns with less than 40% of missing data.

Table 2.

Missing data range	Arctiina	Calisto	Choreutidae	Coenonymphina	Geometridae	Morpho	Noctuidae	Pieridae
0-10%	11%	10%	37%	47%	10%	16%	45%	28%
10-20%	10%	1%	26%	46%	26%	26%	22%	16%
20-30%	14%	5%	1%	5%	29%	20%	12%	35%
30-40%	22%	33%	27%	1%	15%	8%	4%	8%
40-50%	12%	24%	2%	0%	5%	15%	9%	3%
50-60%	17%	22%	3%	0%	3%	3%	5%	5%
60-70%	2%	2%	4%	0%	13%	6%	1%	2%
70-80%	13%	2%	0%	0%	1%	6%	1%	2%
80-90%	0%	0%	0%	1%	0%	0%	0%	0%
90-100%	0%	0%	0%	0%	0%	0%	1%	0%
Cumulative missing data								
0-10%	11%	10%	37%	47%	10%	16%	45%	28%
0-20%	21%	11%	62%	93%	36%	42%	67%	44%
0-30%	34%	16%	63%	97%	64%	62%	79%	79%
0-40%	56%	50%	90%	99%	79%	70%	84%	88%
0-50%	68%	74%	93%	99%	84%	85%	92%	91%
0-60%	85%	96%	96%	99%	87%	88%	97%	96%
0-70%	87%	98%	100%	99%	99%	94%	98%	98%
0-80%	100%	100%	100%	99%	100%	100%	99%	100%
0-90%	100%	100%	100%	100%	100%	100%	99%	100%
100%	100%	100%	100%	100%	100%	100%	100%	100%

Table 3 (on next page)

List of partitioning strategies evaluated for each of the analysed datasets.

TIGER refers to the program that assigns each site in the alignment a relative evolutionary rate, and d is the division factor in the RatePartitions script used to group sites into subsets based on their relative evolutionary rates. See text for more details.

Table 3.

Partitioning strategy	Description
Gene	each gene fragment as separate subset
GeneGr	as above but with PF greedy algorithm combined into similar subsets
Codon	each codon position of each gene as separate subset
CodonGr	as above but with PF greedy algorithm combined into similar subsets
TIG1.5	TIGER partitioning strategy with d=1.5
TIG2.0	TIGER partitioning strategy with d=2.0
TIG2.5	TIGER partitioning strategy with d=2.5
TIG3.0	TIGER partitioning strategy with d=3.0
TIG3.5	TIGER partitioning strategy with d=3.5
TIG3.5Gr	as above but with PF greedy algorithm combined into similar subsets
TIG4.0	TIGER partitioning strategy with d=4.0
TIG4.0Gr	as above but with PF greedy algorithm combined into similar subsets
TIG4.5	TIGER partitioning strategy with d=4.5
TIG4.5Gr	as above but with PF greedy algorithm combined into similar subsets

Table 4(on next page)

The number of partitions for each dataset and partitioning strategy.

Gene refers to partitioning by gene fragment, *Codon* to partitioning by codon position, and *TIG* to partitioning by relative evolutionary rate as estimated with the program TIGER with different values for the d , division factor in the RatePartitions script. See Table 3 and text for more details.

Table 4.

Partitioning strategy	Arctiina	Calisto	Choreutidae	Coenonymphina	Geometridae	Morpho	Noctuidae	Pieridae
Gene	8	6	8	5	8	8	8	8
GeneGr	3	4	4	4	6	4	6	5
Codon	24	18	24	15	24	24	24	24
CodonGr	9	7	10	9	15	7	12	12
TIG1.5	4	4	3	4	7	2	4	6
TIG2.0	5	6	5	5	10	3	6	8
TIG2.5	7	7	6	7	13	4	8	11
TIG3.0	8	9	7	8	15	5	9	13
TIG3.5	10	10	8	9	18	5	11	16
TIG3.5Gr	6	5	6	6	12	4	7	10
TIG4.0	11	12	9	11	21	6	13	18
TIG4.0Gr	5	6	6	7	12	4	6	9
TIG4.5	12	13	10	12	24	7	14	20
TIG4.5Gr	5	7	6	7	16	4	6	8