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RH: Inapplicable data in phylogenetics

Morphological phylogenetic analysis with inapplicable data

MARTIN D. BRAZEAU^{1,2}, THOMAS GUILLERME^{1,3}, MARTIN R. SMITH^{4,5}

¹*Department of Life Sciences, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot SL5 7PY, United Kingdom.*

²*Department of Earth Sciences, Natural History Museum, Cromwell Road, London, SW75BD, United Kingdom.*

³*School of Biological Sciences, University of Queensland, St. Lucia, Queensland, Australia.*

⁴*Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EQ, United Kingdom.*

⁵*Department of Earth Sciences, Mountjoy Site, Durham University, South Road, Durham DH1 3LE, United Kingdom.*

* **Corresponding author.** *m.brazeau@imperial.ac.uk*

All authors contributed equally to this work.

Abstract

1
2 Non-independence of characters is a real phenomenon in phylogenetic data
3 matrices, even though phylogenetic reconstruction algorithms generally assume
4 character independence. In morphological datasets, the problem results in
5 characters that cannot be applied to certain terminal taxa, with this inapplicability
6 treated as “missing data” in a popular method of character coding. However, this
7 treatment is known to create spurious tree length estimates on certain topologies,
8 potentially leading to erroneous results in phylogenetic searches. Here we present a
9 single-character algorithm for ancestral states reconstruction in datasets that have
10 been coded using reductive coding. The algorithm uses up to four traversals on a
11 tree to resolve final ancestral states – which are required in full before a tree can be
12 scored. The algorithm employs explicit criteria for the resolution of ambiguity in
13 applicable/inapplicable dichotomies and the optimization of missing data. We
14 score trees following a previously published procedure that minimizes homoplasy
15 over all characters. Our analysis of published datasets shows that, compared to
16 traditional methods, our new method identifies different trees as “optimal”; as such,
17 correcting for inapplicable data may significantly alter the outcome of tree searches.

18 (Keywords: cladistic analysis, inapplicable data, character independence, phylogenetic
19 tree search, character optimization)

INTRODUCTION

20
21 Morphological characters are an essential source of data in phylogenetic studies. Even
22 though they have been outpaced in their use by molecular sequence data, they remain
23 indispensable for a range of research programmes that depend on knowledge of extinct
24 or ancestral phenotypic conditions (e.g. palaeontology, molecular clock calibrations,
25 comparative developmental biology). Despite advances in the use of probabilistic
26 models for analysing morphological data (e.g. Lewis, 2001; Wright et al., 2016), all
27 transformation-based methods (e.g. parsimony, likelihood) are subject to the same
28 persistent problem in morphological and phenotypic data: the logical inapplicability of
29 characters.

30 Logical inapplicability occurs when a dataset contains characters that can only
31 have a meaningful value in a subset the taxa under investigation. This usually arises
32 when one or more characters are ontologically dependant on a neomorphic
33 (presence/absence) character, here termed the “principal character”. The problems
34 associated with coding these character relationships have been discussed in detail since
35 the advent of desktop phylogenetic computer programs (Farris, 1988; Platnick et al.,
36 1991; Maddison, 1993; Wilkinson, 1995; Pleijel, 1995; Strong and Lipscomb, 1999;
37 Hawkins, 2000; Forey and Kitching, 2000; Fitzhugh, 2006; Brazeau, 2011) and reflect the
38 mathematical consequences of several popular coding procedures (reviewed by
39 Brazeau, 2011). As it stands, no existing software can accommodate the computational
40 issues that arise from logical inapplicability.

41 For situations in which a the state of a character depends on the presence or
42 absence of another character, there is widespread agreement that the best practice is to
43 code inapplicable taxa using a token treated as “missing data”. The token “-” is often
44 used to distinguish cases in which a character is inapplicable (e.g. tail colour, in a taxon
45 that lacks a tail) from those in which a character’s state is uncertain (typically denoted
46 “?” – e.g. tail colour, where the colour of the tail is unknown). For the tail example, this
47 looks like:

- 48 • 1. Tail: absent (0); present (1)
- 49 • 2. Tail colour: blue (0); red (1); inapplicable (? or -)

50 This coding style, termed reductive or contingent coding (Strong and Lipscomb,
51 1999; Forey and Kitching, 2000, hereafter “reductive coding”), treats inapplicable state
52 values as missing data – as though the characteristic in question is not preserved in
53 known specimens. This approach is considered unlikely to lead to implicit (and
54 unintended) character weighting, but does entail spurious calculations (Maddison,
55 1993): such a coding scheme will allow the reconstruction of transformations at nodes
56 where the inferred state is logically impossible (e.g. a change in tail colour in an
57 ancestor with no tail). These logically impossible state reconstructions and their
58 concomitant transformations have been informally referred to as “pseudo-parsimony”,
59 but could be generalized to “pseudo-optimality”, since they would occur in
60 probabilistic calculations as well. In spite of the problem of logically impossible state
61 reconstructions, reductive coding is still widely used and defended (Strong and

62 Lipscomb 1999; Hawkins 2000; Brazeau 2011; but see also arguments from Fitzhugh
63 2006 and Vogt 2017).

64 Maddison (1993) concluded that addressing this problem would require
65 modification of phylogenetic software; 25 years later, there are still few signs of
66 progress on this problem. Some recent and important theoretical advances were made
67 by De Laet (2005, 2015), but De Laet does not describe a single-character algorithm; nor
68 does he provide details of how his method might handle ambiguity, polymorphism,
69 missing data, or multistate characters.

70 In this paper we detail modifications required to enable the Fitch algorithm to
71 process morphological characters that exhibit inapplicability. We consider how trees
72 should be evaluated for optimality, consistent with the method described by De Laet
73 (2015). Furthermore, we show that the effect of “pseudo-optimal” reconstructions can
74 lead to both significant over- and under-estimates of parsimony scores during tree
75 searches. Our algorithm and its implementation allow a special token to indicate
76 inapplicability, meaning that existing datasets that use the gap token to denote
77 inapplicability can be treated with little modification. However, we show that
78 investigators may wish to re-code some characters in ways that can avoid the
79 inapplicable token altogether.

80 THEORETICAL CONSIDERATIONS OF ANCESTRAL STATE 81 RECONSTRUCTIONS AND TREE SCORES

82 We wish to have an algorithm that: (i), incorporates all phylogenetically relevant

83 information; (ii), generates logically and internally consistent nodal state sets, including
84 the reconstruction of an inapplicable “state” where a character does not logically apply;
85 and (iii), calculates exact optimality scores that neither over- nor under-penalize any
86 given tree. In order to reconstruct ancestral states in an ontologically dependent
87 character, it is first necessary optimize the presence or absence of the principal
88 character, which dictates the nodes at which the ontologically dependent character is
89 applicable. This opens up two questions: how do we resolve ambiguity in the principal
90 character, and how do we calculate an optimality metric for the tree?

91 *Ancestral state reconstructions*

92 It is not unusual for a character to have two mutually exclusive nodal reconstruction
93 sets that are equally parsimonious. Particularly relevant here is the case of a
94 presence/absence character whose distribution can be accommodated by one of two
95 equally parsimonious explanations: the gain and loss of the character (accelerated
96 transition / ACCTRAN), or two parallel gains (delayed transition / DELTRAN).

97 By minimizing the number of independent origins in the neomorphic character,
98 the ACCTRAN optimization maximises the homology represented by that character. This
99 is preferable – on the principle of maximising homology and minimising homoplasy
100 (De Laet, 2005) – to the DELTRAN optimization (de Pinna, 1991), in which each
101 independent gain of the character represents an additional instance of homoplasy.

102 Even though the neomorphic character makes an equal contribution to tree
103 length under either reconstruction, the contribution of any ontologically dependent

104 characters may depend on the optimization chosen. By way of example, an ACCTRAN
105 optimization that reconstructs the gain and loss of a red tail implies that all tails are
106 homologous; there is therefore no homoplasy in the ontologically dependent character
107 “tail colour”. In contrast, a DELTRAN optimization that invokes two parallel gains of a
108 red tail on the same tree may be equally parsimonious with respect to the principal
109 character, but would imply two independent origins of red colouration, one of which
110 represents an instance of homoplasy.

111 A satisfactory method must therefore distinguish the presence of a character
112 from its absence: something that is impossible within the Fitch algorithm, which is
113 blind to whether tokens denote presence, absence, or some other property, and
114 therefore cannot differentiate gain/loss from parallel gain. Thankfully, the presence or
115 absence of a principal character is implicit in the distinction between applicable and
116 inapplicable states in an ontologically dependent character. If a dataset is coded
117 accurately, then the applicable/inapplicable distinction will exactly mirror the
118 presence/absence distribution of its principal character. That means that knowledge of
119 presence/absence can be built into the handling of ontologically dependent characters,
120 and that the algorithm need not be explicitly supplied with a prior specification of the
121 principal character.

122 *Scoring trees*

123 For the purpose of phylogenetic searches, there must be some function for evaluating
124 the amount of homoplasy implied by the optimal character reconstructions on a

125 particular tree topology. When all characters are applicable to all terminal taxa, the
126 amount of homoplasy is simply equal to the number of transformations minus the
127 theoretical minimum number of transformations over all characters. However, a
128 transformation between applicable and inapplicable states has no clear meaning with
129 respect to length counts (i.e. it is not an *independent* transformational event).

130 The problem is most clearly illustrated in the context of a principal character
131 with a number of ontologically dependent transformational characters (Fig. 1). If each
132 transformation from an applicable to an inapplicable state contributes to tree length,
133 the loss of the principal character will be severely penalised, even though it can be
134 explained as a single evolutionary event. In contrast, if transformations between
135 applicable and inapplicable states contribute nothing to tree length, then losses and
136 gains of the principal character are inadequately penalised, effectively resulting in a
137 penalty for character congruence, and thus a penalty for homology.

138 This illustrates why the amount of homoplasy within a tree cannot simply be
139 expressed in terms of the number of transformational events when ontologically
140 dependent characters are present (De Laet, 2005, 2015). To once again borrow
141 Maddison's (1993) example, a single transformation from "tail absent" to "tail present,
142 red" does not represent an instance of homoplasy for the ontologically dependant
143 character "tail colour", but if this same transformation happens twice, homoplasy in
144 tail colour has occurred: the tree should be penalized once for the independent origin
145 of the second tail, and once more because the second tail, when it appeared, happened

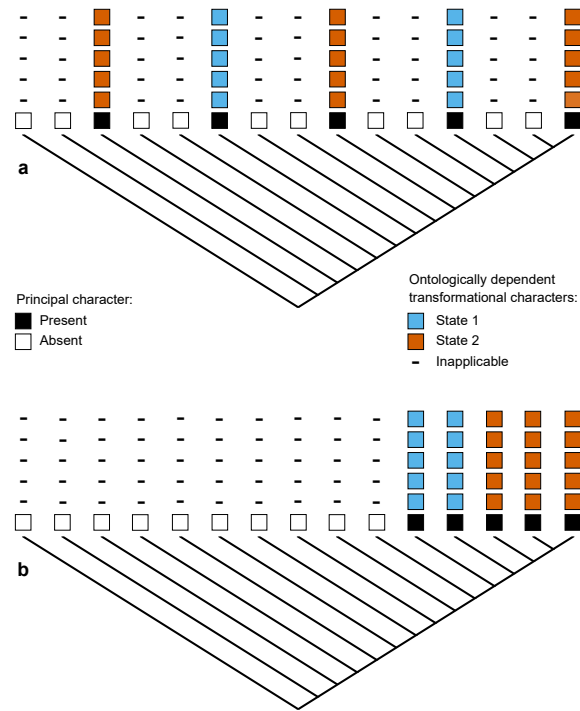


Figure 1: Effect of counting method on tree preference. If transformations between losses and gains of the principal character are inadequately penalised, then trees with multiple gains of the principal character (a) will be favoured; if transformations between applicable and inapplicable states are penalized, then trees in which the principal character evolves exactly once (b) will be favoured.

146 to exhibit the same state (red) as the first (Fig. 2). However, the loss of a tail implies the
147 simultaneous loss of colour and other similar attributes, which cannot similarly be
148 explained as transformations.

149 A satisfactory handling of inapplicable data in morphology must satisfy at least
150 two criteria: (i), non-redundancy; and (ii), maximizing the explanatory value of the data
151 (De Laet, 2005). This is not possible with currently implemented algorithms. De Laet
152 (2005) proposes a solution in which the penalty on the tree is not simply the sum of
153 steps, but also the number of regions (or “subcharacters”) defined by a character.
154 Regions are defined as subtrees in which a character is logically applicable (i.e.
155 applicable character regions). De Laet proposes that the optimal tree is that which
156 minimizes the sum of the number of regions and the number of transformations
157 between states.

158 Throughout this manuscript we therefore make a clear distinction between tree
159 length and tree score, either of which might be chosen as an optimality criterion. Tree
160 length designates the number of transformational events (steps) implied by a topology,
161 whereas tree score designates an optimisation value that combines some function of the
162 tree length with other non-transformational events, such as the sum of the number of
163 regions.

164 FITCH PARSIMONY WITH PARTIALLY APPLICABLE CHARACTERS

165 The algorithm described below is a single-character (*sensu* Ronquist, 1998) method in
166 which “inapplicability” is reserved as a special token (usually denoted with the symbol

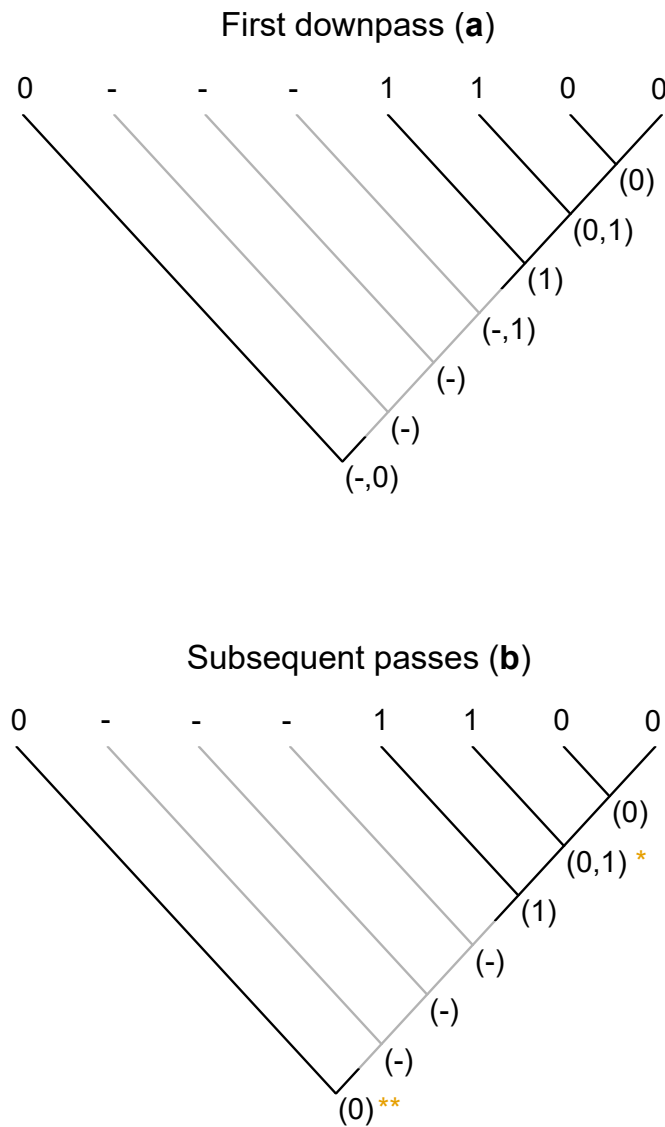


Figure 2: Scoring of a simple tree with inapplicable data. A principal character is present in two regions of the tree (black lines). A transformation from state 1 to state 0 adds one step to tree length. A second occurrence of state 0 represents a case of homoplasy, and should also contribute to tree score. In our algorithm, the first downpass (a) generates possible reconstructions of each node; final state reconstructions are generated in the first uppass and not modified by further passes. The second downpass increments tree length by two, reflecting one step (at *) and one additional region (at **).

167 “-”). At its core, the algorithm resembles the Fitch algorithm (Fitch, 1971), with
168 “inapplicability” being treated as an additional state. The first two passes of the
169 algorithm use the distribution of applicable and inapplicable tokens to infer whether
170 the associated principal character can be optimally reconstructed as present at each
171 node. In nodes where the principal character can be reconstructed as present, normal
172 Fitch rules are used to identify and count transformations. Transformations are not
173 reconstructed at nodes where the principal character was necessarily absent.

174 To count the number of applicable regions, a flag is stored at each node which
175 records whether or not any descendants store applicable values. This allows the
176 number of regions to be incremented when moving, on the second downpass or on the
177 second uppass, from an inapplicable region of the tree to an applicable region. Up to
178 four passes are therefore required to complete ancestral state reconstructions for a
179 given node (Fig. 3). An interactive visualisation of the four passes is available via the
180 Inapp R package at <https://github.com/TGuillerme/Inapp>.

181 *First postorder traversal (downpass) – Figs 2a, 3a*

182 Traverse the internal nodes of the tree in postorder. At each node:

- 183 1. **If** there is any token in common between both descendants, *go to 2*; **else** *go to 3*.
- 184 2. **If** the token in common is only the inapplicable token, and both descendants have
185 an applicable token, *set* the node’s state to be the union of the descendants’ states;

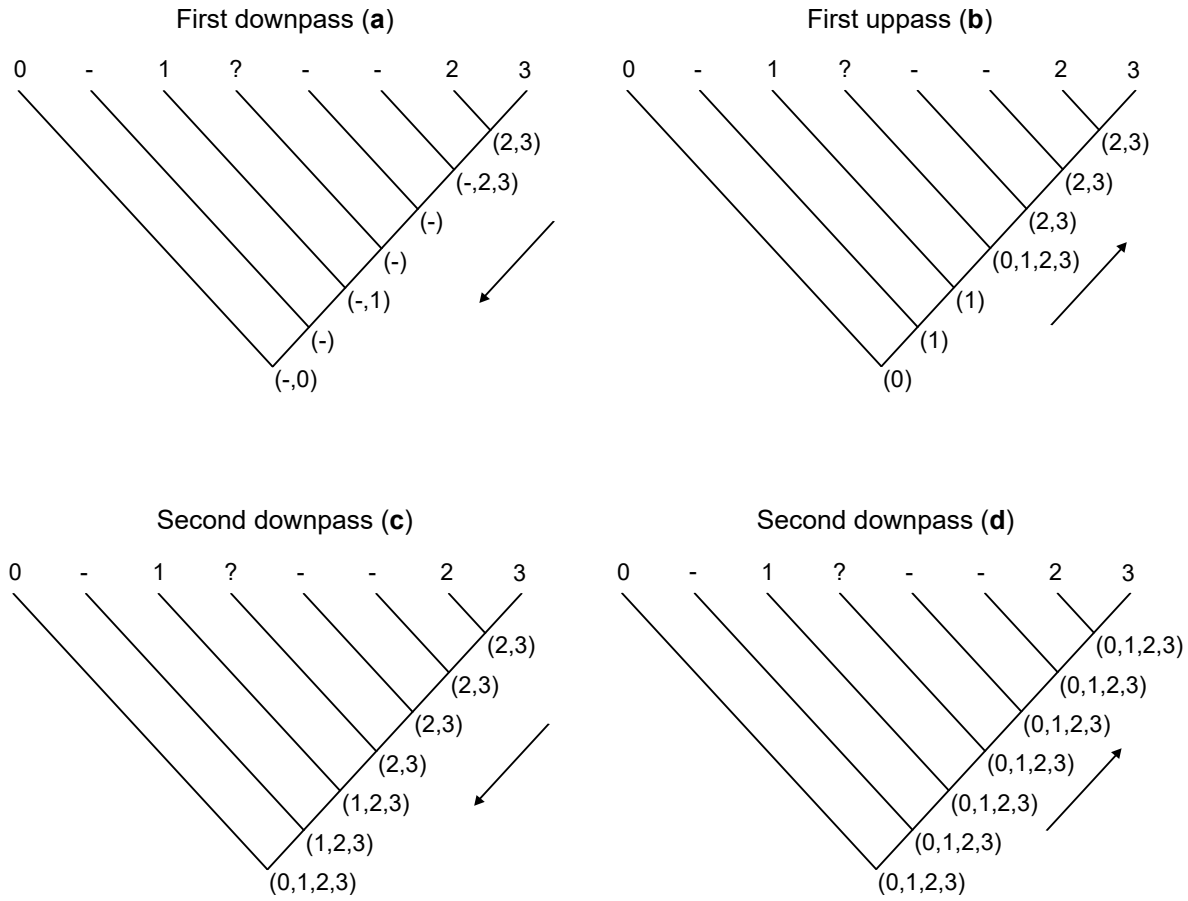


Figure 3: Illustration of the use of four passes for correctly estimating the ancestral states with inapplicable data in this phylogeny. After the second pass, the ancestral state sets are incorrect at six of the seven nodes.

- 203 5. **If** any of the descendants have an applicable token, *set* the node's state to be the
204 union of the applicable states of its descendants; **else** *set* the node's state to be the
205 inapplicable token only. **Then** *go* to 8.
- 206 6. **If** the unvisited tip includes both inapplicable and applicable tokens, *go* to 7; **else**
207 *go* to 8
- 208 7. **If** the current node has only the inapplicable token, *set* the tip's state to the
209 inapplicable token only; **else** *remove* the inapplicable token from the tip's state.
210 **Then** *go* to 8.
- 211 8. **If** one of the node's descendants is an unvisited tip, *go* to 6; **else** visit the next
212 node in preorder. Once all nodes and tips have been visited, *initialise the tracker*.

213 *Initialise tracker – Figs. 2b, 3c*

214 Visit each tip in turn. At each tip:

- 215 1. **If** the tip's state contains the inapplicable token, *set* its tracker to "off" and *go* to 4;
216 **else** *go* to 2.
- 217 2. **If** the tip's state does not contain the inapplicable token, *set* its tracker to "on" and
218 *go* to 4; **else** *go* to 3.
- 219 3. **If** the ancestor's state contains an inapplicable token, *set* the tip's tracker to "off";
220 **else** *set* the tip's tracker to "on". **Then** *go* to 4.

221 4. Visit the next tip. Once all tips have been visited, *conduct the second downpass*.

222 *Second postorder traversal (downpass) – Figs. 2b, 3c*

223 Traverse the tree in postorder. At each node:

224 1. **If** the tracker of either descendant is “on”, *set* this node’s tracker to “on”; **else set**
225 it to “off”. **Then**, *go* to 2

226 2. **If** the node had an applicable token in the first uppass, *go* to 3; **else** leave the
227 node’s state unchanged and *go* to 8.

228 3. **If** there is any token in common between both descendants, *go* to 4; **else** *go* to 5.

229 4. **If** the tokens in common are applicable, *set* the node’s state to be the tokens held
230 in common, without the inapplicable token; **else set** the node’s state to be the
231 inapplicable token. **Then** *go* to 8.

232 5. *Set* the node’s state to be the union of the states of both descendants (if present)
233 without the inapplicable token, and *go* to 6.

234 6. **If** both descendants have an applicable token, *increment* the tree score (step
235 increment) and *go* to 8; **else** *go* to 7.

236 7. **If** both of the node’s descendants’ trackers are “on”, *increment* the tree score
237 (applicable region increment) and *go* to 8; **else** just *go* to 8.

238 8. Visit the next node in postorder. Once all nodes have been visited, *conduct the*
239 *second uppass.*

240 *Second preorder traversal (uppass) – Figs. 2b, 3d*

241 Traverse the tree in preorder. At each node:

- 242 1. **If** the node has any applicable token, *go to 2*; **else go to 9.**
- 243 2. **If** the node's ancestor has any applicable token, *go to 3*; **else go to 10.**
- 244 3. **If** the node's state is the same as its ancestor's, *go to 10*; **else go to 4.**
- 245 4. **If** there is any token in common between the node's descendants, *go to 5*; **else go**
246 **to 6.**
- 247 5. *Add* to the current node's state any token in common between its ancestor and its
248 descendants and *go to 10.*
- 249 6. **If** the states of the node's descendants both contain the inapplicable token, *go to 7*;
250 **else go to 8.**
- 251 7. **If** there is any token in common between either of the node's descendants and its
252 ancestor, *set* the node's state to be its ancestor's state; **else set** the current node's
253 state to be all applicable tokens that are common to both its descendants and
254 ancestor. **Then go to 10.**
- 255 8. *Add* to the node's state the tokens of its ancestor. **Then go to 10.**

- 256 9. If both of the node's descendants' trackers are "on", *increment* the tree score
257 (applicable region increment) and *go* to 8; **else** *go* to 10.
- 258 10. Visit the next node in preorder. Once all nodes have been visited, *calculate the tree*
259 *score*.

260 *Calculate tree score*

261 The contribution of the given character to the total score of the tree is given by:

$$\text{Contribution to tree score} = \text{number of state changes} + \text{number of additional regions} \quad (1)$$

262 State changes are recorded in the second downpass (at point 6); the number of
263 applicable regions is calculated in both the second downpass (at point 7) and the
264 second uppass (point 9).

265 CHARACTER CODING WITH INAPPLICABLE-AWARE 266 RECONSTRUCTION ALGORITHMS

267 *Two categories of ontologically dependent character*

268 An upshot of recognizing maximized homology and minimized homoplasy as the
269 objective of maximum parsimony is that not all cases of ontological dependency of
270 characters (*sensu* Vogt, 2017) require reductive coding. Two coding strategies may be

271 applied, depending on the *information* implied by the states of an ontologically
272 dependent character. That is, we may distinguish between subcharacters that are
273 *transformational* (as in the case of tail colour) and *neomorphic* (following Sereno, 2007).

274 Transformational character statements describe a variable property of a principal
275 character, with no biological reason to anticipate any particular ancestral state. The case
276 of tail colour, as discussed above, is transformational; with reductive coding, it can be
277 correctly handled by our algorithm. If a red tail appears twice on a particular tree
278 topology, then the fact that it is red in both instances represents an instance of
279 homoplasy: an independent innovation of the colour red. Using the inapplicable token
280 to denote tail colour in non-tail-bearing taxa (Table 1) causes our algorithm to recognize
281 the second innovation of a red colouration as an instance of homoplasy that should
282 contribute to the tree's length.

283 Neomorphic character statements are presence/absence characters that depend
284 on the presence of the principal character. An example would be the presence of
285 eyespots on a tail. Such characters may be scored as binary characters without the use
286 of the inapplicable token, as long as there is still a separate character for
287 presence/absence of tail. Given the presence of a tail, a researcher might conclude that
288 the absence of eyespots, or equivalent features, is unsurprising. Two separate instances
289 of a tail without eyespots would then be said to exhibit a homoplasy with respect to tail
290 presence, but not with respect to the absence of eyespots. Unlike the case of tail colour
291 (a tail must have *some* colour when it appears), the presence of an eyespot is not

Tail	0	0	0	1	1	1	1	1	(0, absent; 1, present)
Tail colour	-	-	-	0	0	1	2	?	(0, red; 1, blue; 2, green; -, inapplicable)
Tail eyespot	0	0	0	1	0	1	0	?	(0, absent; 1, present)

Table 1: Coding inapplicable data in ontologically dependent characters. “Tail” is a principal character with two ontologically dependent characters, “Tail colour”, a transformational character that should be coded as “-” when a tail is absent, and “Tail eyespot”, a neomorphic character that should be coded as “o” when a tail is absent.

292 necessarily expected. The absence is an uninformative value, and therefore would be
293 more difficult to describe as homoplasious. If, on the other hand, eyespots are present
294 on the two occasions that a tail appears, then the second occurrence of eyespots does
295 represent an instance of homoplasy. Likewise, a secondary *loss* of eyespots elsewhere
296 on the tree would represent an instance of convergence and should therefore contribute
297 to tree length. For this reason, simple binary presence/absence coding may be
298 employed, where an absence value would cover both observed absence and absence
299 due to the absence of the principal character (Table 1). Even when applying our
300 algorithm, inapplicable tokens should not be employed in such instances, as they
301 would incorrectly penalize trees in which a tail (lacking eyespots) originated multiple
302 times. If additive characters are decomposed into a series of neomorphic characters,
303 then the original and decomposed characters are mathematically equivalent under this
304 coding approach.

305 *“Parsimony-uninformative” characters inform parsimony*

306 A consequence of our approach is that the distribution of inapplicable tokens conveys
307 grouping information. A topology that implies that red tail colouration evolved once
308 has a shorter length than one on which red tail colouration evolved twice, even if no
309 other colour of tail is observed. This seemingly counterintuitive result arises because
310 the algorithm prefers trees that attribute similarities to common ancestry rather than to
311 chance. The effect of including ontologically dependent characters that would not be
312 parsimony-informative under the standard Fitch algorithm is to up-weight the
313 corresponding principal character. Care must be taken, therefore, that each
314 ontologically dependent character truly reflects a biologically significant similarity, for a
315 principal character might be misleadingly upweighted if trivial subordinate properties
316 (e.g. “number of DNA bases in tail”) are included in a matrix.

317 *Missing but applicable character states*

318 With an implementation of the Fitch algorithm that does not consider the inapplicable
319 token as equivalent to missing data, extra care should be employed when coding
320 missing data. Consider an example dataset that includes fossil and extant species.
321 Following the character “Tail colour” described above (Table 1), one could code a fossil
322 taxon where the tail is entirely missing due to incomplete preservation. In this case, the
323 “Tail colour” should be coded as “?” (it is uncertain whether the tail was red, blue or
324 green or whether the tail was present at all). If we now consider another fossil taxon

325 were the tail is clearly preserved but the colour is not observable, the character state
326 ambiguity could be coded in one of three ways:

- 327 • If, as is the usual case, there is no *a priori* information indicating whether or not a
328 tail is homologous with those of other taxa, tail colour should be coded as
329 uncertain (“?”), treated by the algorithm as (-012).

- 330 • If the tail of the fossil taxon is known to be homologous with the tails of other
331 taxa, then an optimal character reconstruction will assert that its colour is one of
332 the colours that has been observed in other taxa (the ambiguity should be coded
333 as “red, blue or green” (012)).

- 334 • If the tail of the fossil taxon is known *not* to be homologous with the tails of other
335 taxa, then an optimal character reconstruction will assert that its colour is *different*
336 from any colour that has been observed, because a second innovation of a
337 colouration observed elsewhere on the tree would represent an instance of
338 homoplasy. In this case, tail colour should be coded as inapplicable (-), the
339 character’s definition being effectively “colour of tail of homologous type”.

340 COMPARING APPROACHES TO PHYLOGENETIC RECONSTRUCTION

341

342 In order to evaluate how this approach impacts phylogenetic results, we analyzed 30
343 discrete morphological matrices under three approaches: (i), reductively coded datasets

344 treated under traditional Fitch parsimony, with inapplicable treated as missing (here
345 termed the “missing” approach); (ii), the “extra state” approach, using compound
346 coding with inapplicability as a separate state; and (iii), the “inapplicable” approach,
347 which applies our new algorithm.

348 Before beginning our analysis, every inapplicable token in each neomorphic
349 character was replaced with the token corresponding to the presumed non-derived
350 condition (typically “absent”). Each matrix was then subjected to phylogenetic tree
351 search: the “missing” and “extra state” approaches used TNT, employing the parsimony
352 ratchet, sectorial search and tree drifting algorithms (Goloboff, 1999; Nixon, 1999;
353 Goloboff and Catalano, 2016); the inapplicable approach used R (R Core Team, 2017) for
354 tree search, using the parsimony ratchet as deployed in our new package *inapplicable*
355 (see Implementation section below). Although this latter search approach is inefficient,
356 it nevertheless converges on an optimal tree length within minutes (<50 tips) to hours
357 (<80 tips). Whilst it is difficult to guarantee that every optimal tree will be identified,
358 we ensured a wide sampling of tree space by conducting 100 independent tree searches
359 in TNT, and by sampling shortest trees in R until the shortest length had been found by
360 250 ratchet iterations.

361 In order to establish whether the three methods recovered different sets of
362 optimal trees, the number of trees that occurred in the optimal sets of one, two, or all
363 three approaches was tallied. In addition, a strict consensus tree was calculated for all
364 trees in each optimal set, the number of bipartitions present in each set serving as a

365 proxy for the disparity of trees that is optimal under each approach. Finally, each set of
366 optimal trees was plotted in a two-dimensional space (Hillis et al., 2005) by
367 decomposing a matrix of pairwise quartet distances (Estabrook et al., 1985), calculated
368 using the `tqDist` R library (Sand et al., 2014), into two dimensions by minimising the
369 Kruskal-1 stress function (Borg and Groenen, 2005), following Hillis et al. (2005).

370 *Results*

371 In most cases, the three different methods identified different sets of optimal trees.
372 Indeed, only in one of the thirty examined datasets were the optimal trees recovered by
373 each method also optimal under the other two (Fig. 4a). In ten datasets (Fig. 4b), a
374 subset of trees are optimal under all methods, but other trees are optimal under one
375 method and a few steps longer under another. In nine datasets (Fig. 4c), the forests of
376 trees that are optimal under two methods (here, “missing” and “extra state”) partially
377 overlap, but in one method (here, “inapplicable”), no optimal trees were found that are
378 also optimal under either other method. In the final ten datasets (Fig. 4d), each method
379 generates a distinct set of optimal trees. Summing across all datasets, only 4% of trees
380 that were optimal under one method were also optimal under the other two (Fig. 5a).

381 How topologically different were the trees that each method described as
382 optimal? One qualitative way to explore the difference between multiple forests of trees
383 is to generate a two-dimensional treespace from the distances between pairs of trees.
384 This approach demonstrates that it is difficult to predict which methods will identify



x axis: score over method's optimum, when trees in forest are scored under:

■ 'ambiguous' ■ 'extra state' ■ 'inapplicable'

Figure 4: Different methods recover different optimal tree sets. Each histogram details the distribution of tree scores when a each of the optimal trees recovered under method P is scored using method Q. Scores are presented relative to the lowest score recovered by method Q for each dataset. Histograms for all examined datasets are presented in the supplementary information.

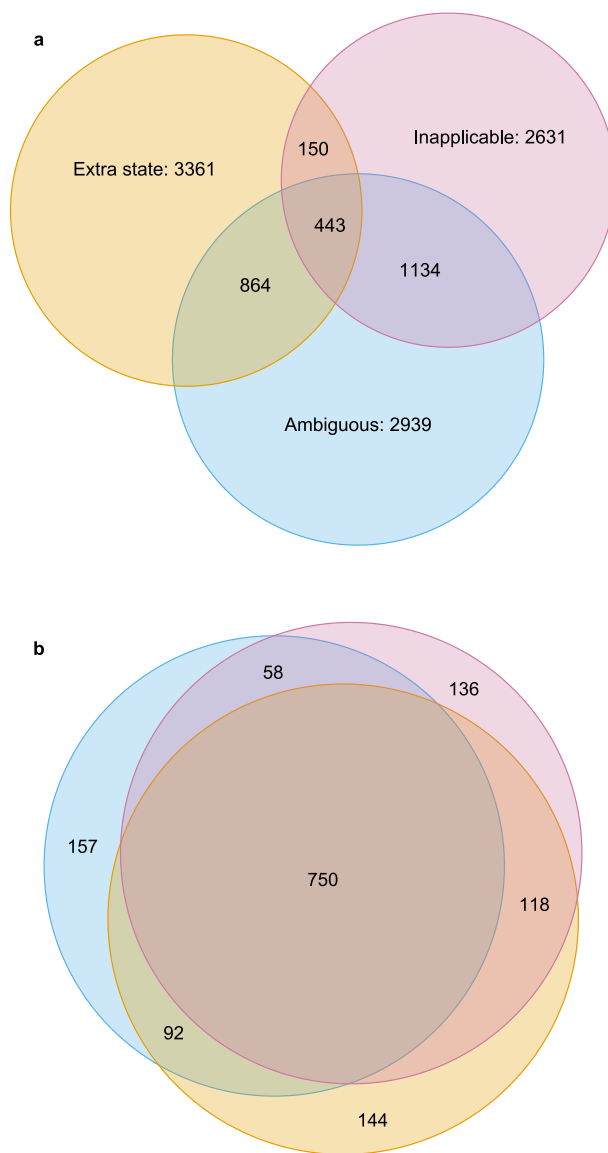


Figure 5: Venn diagrams depicting (a), proportions of optimal trees that are optimal under one, two or three methods; (b), proportion of nodes present in every optimal tree recovered under one, two or three methods. Results are summed across all datasets; figures for individual datasets are available in the supplementary information.

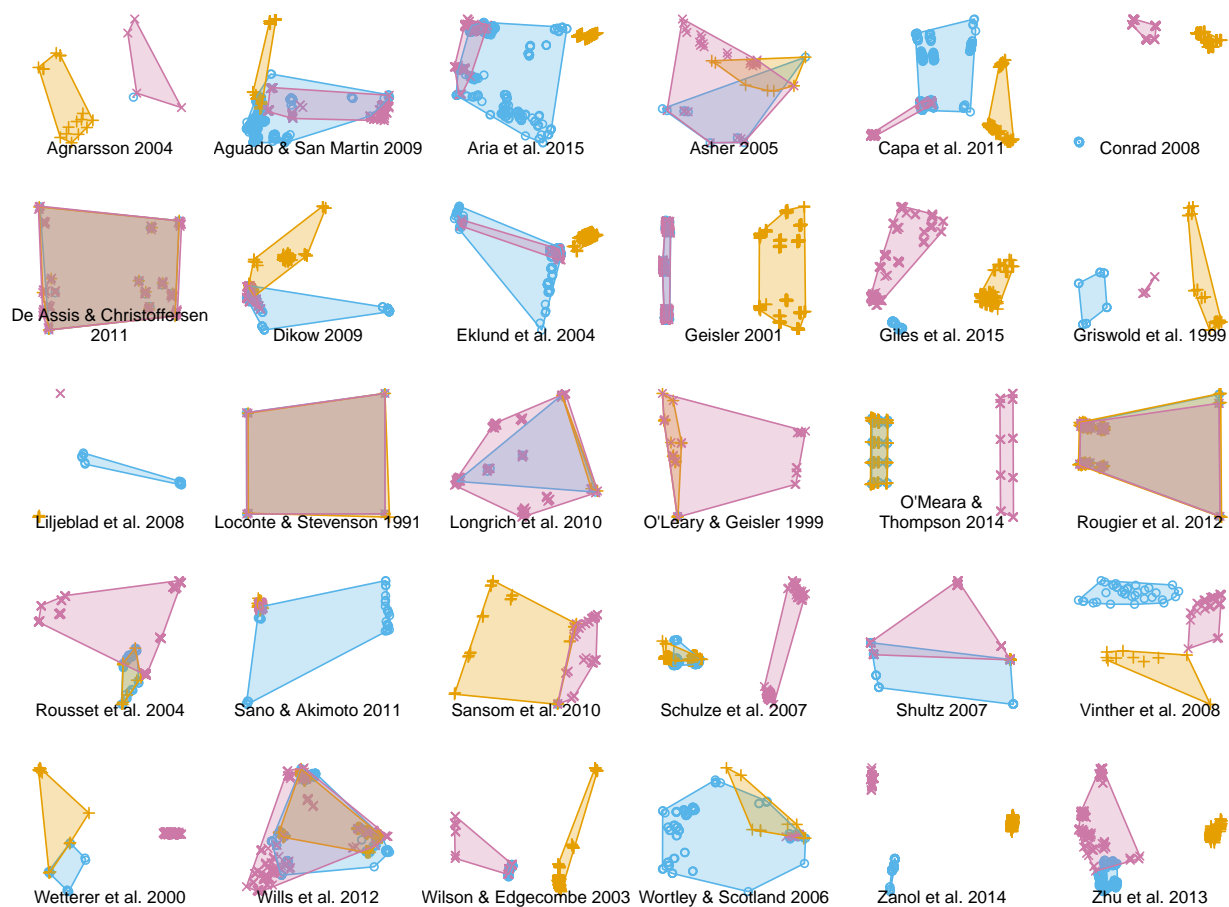


Figure 6: Distribution of optimal trees in MDS treespace for each dataset. Shaded regions correspond to convex hulls surrounding all optimal trees recovered using a given approach. No method is consistently more precise or more similar to any other method.

385 the most similar sets of optimal trees, and that the regions of treespace identified as
386 optimal by the different methods may be very different or very similar (Fig. 6).

387 An alternative way to explore how much trees in the three optimal sets have in
388 common is to count the number of nodes held in common between trees within a set –
389 or, in other words, the number of nodes present on the strict consensus of all trees in
390 that set. On this approach, averaged across all datasets, 76% of the nodes that are
391 present in every tree that is optimal under the “inapplicable” approach are also present
392 in every tree that is optimal under the “missing” approach, and 82% are present in
393 every tree that is optimal under the extra-state approach; only 70% are present in all
394 trees recovered by all methods (Fig. 5b).

395 Even though, in any one dataset, the number of trees identified as optimal can
396 vary considerably between the three methods, we were unable to identify any
397 systematic trend in the disparity of optimal trees. Neither the number of distinct trees
398 in the optimal tree set, the resolution of the strict consensus tree, nor the area of
399 treespace occupied by the trees showed any systematic variation.

400 *Implications*

401 The accuracy of a method measures whether the method will reconstruct the “true” tree
402 from a given dataset. As the “true” evolutionary tree is unknown, attempts to measure
403 the accuracy of phylogenetic methods rely on data simulated from a predetermined tree
404 topology. In the absence of a robust and testable model that can realistically simulate
405 inapplicable morphological data, it is not possible to objectively compare the accuracy

426 Furthermore, missing data need to be updated at the tips – initially as either applicable
427 or inapplicable – in order to complete ancestral state sequences. Our tree scoring
428 procedure follows De Laet (2015) in penalizing increasing amounts of homoplasy
429 without redundant penalties. Up to three traversals are necessary in order to count the
430 number of transformations on a tree, which can be achieved during the second
431 downpass. However, a final estimate of the number of regions on the tree is counted on
432 the fourth traversal (final uppass). The method, unsurprisingly, takes additional time,
433 though this is expected to be mostly in proportion to the number of characters having
434 inapplicable tokens. Nevertheless, some economies are possible, because only
435 characters with three or more inapplicable tokens need to be treated with this
436 algorithm. The method provides a means of evaluating existing datasets with minimal
437 modification, and without a need to specify explicit relationships between characters
438 (because presence/absence information is already implicit in the
439 applicable/inapplicable distinction). Preliminary results show that analyses with large
440 amounts of inapplicable data are likely to be considerably affected by inapplicable data.
441 In some cases, the set of trees that are optimal under our new algorithm does not
442 overlap with the optimal sets obtained by existing approaches, suggesting that our
443 method allows a gain in accuracy with no corresponding loss of precision.

444 IMPLEMENTATIONS

445 The algorithm described throughout this paper is implemented at different levels in
446 different projects. The main C implementation of the algorithm and associated tools is

447 available at <http://www.morphyproject.org/>. An R implementation based on the
448 former is available in the `inapplicable` package at
449 <https://github.com/ms609/inapplicable>. Finally, a shiny (R) visualisation of the
450 algorithm is available via the `Inapp` package at
451 <https://github.com/TGuillaume/Inapp>. Permanent archives of the above
452 implementations are available on FigShare,
453 <http://dx.doi.org/10.6084/m9.figshare.c.3911821>

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461 References

462 Borg, I. and P. J. Groenen. 2005. Modern multidimensional scaling: Theory and
463 applications. Springer Science & Business Media.

- 464 Brazeau, M. D. 2011. Problematic character coding methods in morphology and their
465 effects. *Biological Journal of the Linnean Society* 104:489–498.
- 466 De Laet, J. 2005. Parsimony, Phylogeny and Genomics chap. Parsimony and the
467 problem of inapplicables in sequence data, Pages 81–116. Oxford University Press.
- 468 De Laet, J. 2015. Parsimony analysis of unaligned sequence data: maximization of
469 homology and minimization of homoplasy, not minimization of operationally
470 defined total cost or minimization of equally weighted transformations. *Cladistics*
471 31:550–567.
- 472 de Pinna, M. C. 1991. Concepts and tests of homology in the cladistic paradigm.
473 *Cladistics* 7:367–394.
- 474 Estabrook, G. F., F. McMorris, and C. A. Meacham. 1985. Comparison of undirected
475 phylogenetic trees based on subtrees of four evolutionary units. *Systematic Zoology*
476 34:193–200.
- 477 Farris, J. 1988. Hennig86, version 1.5. Distributed by the author, Port Jefferson Station,
478 NY .
- 479 Fitch, W. M. 1971. Toward defining the course of evolution: minimum change for a
480 specific tree topology. *Systematic Biology* 20:406–416.
- 481 Fitzhugh, K. 2006. The requirement of total evidence and its role in phylogenetic
482 systematics. *Biology and Philosophy* 21:309–351.

- 483 Forey, P. L. and I. Kitching. 2000. Experiments in coding multistate characters.
484 Systematics Association Special Volume 58:54–80.
- 485 Goloboff, P. A. 1999. Analyzing large data sets in reasonable times: Solutions for
486 composite optima. *Cladistics* 15:415–428.
- 487 Goloboff, P. A. and S. A. Catalano. 2016. TNT version 1.5, including a full
488 implementation of phylogenetic morphometrics. *Cladistics* 32:221–238.
- 489 Hawkins, J. A. 2000. A survey of primary homology assessment: different botanists
490 perceive and define characters in different ways. Systematics Association Special
491 Volume 58:22–53.
- 492 Hillis, D. M., T. A. Heath, and K. S. John. 2005. Analysis and visualization of tree space.
493 *Systematic biology* 54:471–482.
- 494 Lewis, P. 2001. A likelihood approach to estimating phylogeny from discrete
495 morphological character data. *Systematic Biology* 50:913–925.
- 496 Maddison, W. P. 1993. Missing data versus missing characters in phylogenetic analysis.
497 *Systematic Biology* 42:576–581.
- 498 Nixon, K. C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis.
499 *Cladistics* 15:407–414.
- 500 Platnick, N. I., C. E. Griswold, and J. A. Coddington. 1991. On missing entries in
501 cladistic analysis. *Cladistics* 7:337–343.

502 Pleijel, F. 1995. On character coding for phylogeny reconstruction. *Cladistics* 11:309–315.

503

504 R Core Team. 2017. R: A language and environment for statistical computing .

505 Ronquist, F. 1998. Fast Fitch-parsimony algorithms for large data sets. *Cladistics*

506 14:387–400.

507 Sand, A., M. K. Holt, J. Johansen, G. S. Brodal, T. Mailund, and C. N. Pedersen. 2014.

508 tqdist: a library for computing the quartet and triplet distances between binary or

509 general trees. *Bioinformatics* 30:2079–2080.

510 Sereno, P. C. 2007. Logical basis for morphological characters in phylogenetics.

511 *Cladistics* 23:565–587.

512 Strong, E. E. and D. Lipscomb. 1999. Character coding and inapplicable data. *Cladistics*

513 15:363–371.

514 Vogt, L. 2017. Towards a semantic approach to numerical tree inference in

515 phylogenetics. *Cladistics* .

516 Wilkinson, M. 1995. Coping with abundant missing entries in phylogenetic inference

517 using parsimony. *Systematic Biology* 44:501–514.

518 Wright, A. M., G. T. Lloyd, and D. M. Hillis. 2016. Modeling character change

519 heterogeneity in phylogenetic analyses of morphology through the use of priors.

520 *Systematic Biology* 65:602–611.