Title: Morphological and phylogeographic evidence for budding speciation: an example in hominins
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Abstract

Parametric phylogenetic approaches that attempt to delineate between distinct 'modes' of speciation (splitting cladogenesis, budding cladogenesis, and anagenesis) between fossil taxa have become increasingly popular among comparative biologists. But it is not yet well-understood how clearly limited morphological data from fossil taxa speak to detailed questions of speciation mode as compared to the lineage diversification models that serve as their basis. In addition, the congruence of inferences made using these approaches with geographic patterns has not been explored. Here, I extend a previously introduced maximum-likelihood approach for the examination of ancestor-descendant relationships to accommodate budding speciation and apply it to a dataset of fossil hominins. I place these results in a phylogeographic context to better understand spatial dynamics underlying the hypothesized speciation patterns. Finally, I determine whether instances of budding are driven by morphological versus stratigraphic evidence, or both. The spatial patterns implied by the phylogeny hint at the complex demographic processes underlying the spread and diversification of hominins throughout the Pleistocene. I also find that inferences of budding are driven primarily by stratigraphic, versus morphological, data and discuss the ramifications for interpretations of speciation process in hominins specifically and from phylogenetic data in general.

Introduction

Parametric phylogenetic approaches that aim to distinguish between competing 'modes' of speciation between fossil taxa have rapidly proliferated over the past several years. These approaches typically employ extended models of lineage diversification that allow taxa to be related by one of several possible speciation patterns that have long been discussed by paleontologists (Stadler et al. 2018). These include: 1) splitting cladogenesis, where a lineage splits evenly into two daughter lineages, 2) budding cladogenesis, where a smaller daughter lineage splits off of an older ancestral lineage, and 3) anagenesis, where a single lineage continuously evolves without splitting. These approaches aim to reconstruct ancestor-descendant (AD) sequences of fossil taxa through the stratigraphic record using a unified model of lineage diversification and stratigraphic preservation (Gavryushkina et al. 2014, Zhang et al. 2016). These approaches have been increasingly leveraged to address empirical patterns in the fossil record (Wright et al. 2020), yielding results that authors have interpreted to highlight the ubiquity of budding speciation as a dominant process in the diversification of new taxa (Bapst and Hopkins 2017). Nevertheless, few investigations have directly examined morphological support for mode. The use of geographic data has also remained under-explored (Fisher 1994, Wright 2017).

Hominins are a strong candidate taxon in which to examine alternative speciation modes in the fossil record, with a history of disagreements over evolutionary mode spanning back decades. Early approaches employed cladograms and stratophenetic diagrams to examine relationships (Delson et al., 1977, Gingerich 1979, Chamberlain and Wood 1987). Repeated attempts to reconstruct hominin phylogeny (Wood 1992, Lieberman et al. 1996, Strait et al. 1997, Strait and Grine 2004, Irish et al. 2013) left major controversies concerning specific hypotheses of direct ancestry unresolved (Strait and Wood 1999, Stringer 2012, Gómez-Robles et al. 2013). Parametric approaches have since been applied to hominins, showing promise in quantifying statistical support for competing hypotheses (Dembo et al. 2015). Further explorations have incorporated AD relationships (Parins-Fukuchi et al. 2019). Despite this improved resolution, many important issues remain in hominin phylogeny. Nevertheless, the role of more complex speciation scenarios such as budding have not been statistically evaluated.

In this study, I examine the morphological and phylogeographic evidence for budding speciation in shaping hominin phylogeny. I extended a previous parametric approach for 'stratophylogenetic' inference, including ancestor-descendant relationships, from morphological and stratigraphic data to accommodate budding cladogenesis, which is considered here as a directly ancestral arrangement where a putative ancestor overlaps with a descendant in temporal range. I then use the phylogeny to explore the phylogeographic patterns underlying the spread and morphological divergence of Pleistocene *Homo* populations. Lastly, I evaluate whether morphological data alone support the AD relationships that underpin inferences of budding when the stratigraphic record is not considered.

Methods and materials

Morphometric data: I borrowed a dataset of cranial landmark coordinates from the literature gathered from 18 fossil hominin specimens and two extant outgroup taxa (González-José et al. 2008). For use here, I aligned the 138 3-dimensional landmark coordinates using Procrustes superimposition in MorphoJ (Klingenberg 2011). I then transformed the data using a principal component analysis in R (R Core Team 2014), yielding a dataset of independent variables.

Evaluating ancestor-descendant relationships: I merged and extended existing approaches for parametric phylogenetic inference from continuous traits (Parins-Fukuchi 2018) and the evaluation of ancestor-descendant relationships using discrete traits (Parins-Fukuchi et al. 2019). This approach, implemented as part of the *cophymaru* software package (https://github.com/carolinetomo/cophymaru), automatically rearranges a starting tree to consider hypotheses of direct ancestry between taxa. These perturbations include both traditional 'local' rearrangements (nearest-neighbor interchange) and moves that create direct ancestors. Morphological likelihoods are calculated under a Brownian model (Felsenstein 1973). To provide stratigraphic context, trees are also evaluated against the fossil record using a Poisson model of stratigraphic preservation (Huelsenbeck and Rannala 1997). Since the collapse of terminal taxa into direct ancestors reduces the number of estimated branch lengths, the multiplied morphological and stratigraphic likelihoods are penalized using the Akaike information criterion (AIC). This enables the statistical comparison of trees that differ in dimension. For additional methodological details concerning the use of i criteria, Poisson preservation models, and AD relationships in phylogenenetic reconstruction, see Parins-Fukuchi et al. (2019). The best-supported topology is the one that best represents the morphological data and observed stratigraphic ranges while minimizing superfluous bifurcations between taxa.

The degree of confidence in the placement of each candidate taxon as a direct ancestor on the best-supported stratophylogenetic tree was calculated using AIC weights (Wegenmakers and Farrell 2004). Here, the AIC weight of the ancestral positioning of each taxon was calculated as the proportion of a model 'support surface' occupied by the tree where the candidate is a direct ancestor. The support surface for each putative ancestor was composed of two trees: one with the candidate taxon as an ancestor and one where the candidate taxon is positioned as terminal branch. The positions of the other taxa were held at their maximum-likelihood (ML) AD assignments (positioned as ancestors if the ML tree favored this arrangement, or vice versa). The resulting support value can be interpreted as the model evidence for the assignment of the candidate taxon as a direct ancestor.

Biogeographic reconstruction: I reconstructed biogeographic ranges for internal nodes that did not correspond to a known taxon using the C++ implementation of the lagrange software package (https://github.com/blackrim/lagrange).

Results and Discussion

Budding speciation in hominin evolution: When considered in a stratophylogenetic framework, morphometric and temporal data suggest several possible episodes of budding speciation. Neither of the *Australopithecus* samples included in the analysis were identified as ancestral taxa. Like several previous studies, *A. afarensis* is recovered as an outgroup to a clade comprised of all of the later-occurring hominin morphotaxa. Many authors have interpreted those cladistic results to imply that *A. afarensis* is ancestral to *Homo*. However, these results suggest that *A. afarensis* is not directly ancestral but is instead a terminal outgroup. Since *A. anamensis* appears to be a chronospecies that is ancestral to *A. afarensis* (Kimbel 2006, Parins-Fukuchi et al. 2019), it is possible that the specimens assigned to *A. anamensis* represent a population ancestral to both *A. afarensis* and the later-occurring hominin lineages. *P. boisei* is identified as the direct ancestor of *P. robustus*. This finding is consistent with previous qualitative interpretations (Kimbel 2007). *P. aethiopicus*, on the other hand, is reconstructed with high confidence to be a distinct, branching taxon.

The analysis revealed support for a budding event between *H. erectus* and the clade encompassing *H. heidelbergensis, H. rhodesiensis,* Neanderthals, and modern humans. The mid-Pleistocene specimens corresponding to *H. heidelbergensis* and *H. rhodesiensis* grouped polyphyletically, forming clades with Neanderthals and *H. sapiens*, respectively. This result is notable given the historical tendency to treat specimens corresponding to each taxon (as treated here) as geographic variants of *H. heidelbergensis*. *H. heidelbergensis*, represented by only European specimens in this study, was reconstructed as a budding ancestor to Neanderthals, while *H. rhodesiensis* was reconstructed as a sister taxon to modern humans. The placement of European *H. heidelbergensis* specimens as directly ancestral to Neanderthals is consistent with both the genomic results of Meyer et al. (2016) and the morphological results of Mounier and Caparrós (2016), the former of which inferred the Atapuerca specimens as most closely related to Neanderthals and the latter of which reconstructed both Atapuerca 5 and Steinheim (the two specimens representing *H. heidelbergensis* in this study) as outgroups to Neanderthals. It does however, disagree with a morphospecies-level phylogenetic analysis, which inferred *H. heidelbergensis*, defined as including *both* the European and African samples included here, as ancestral to both Neanderthals and modern humans (Parins-Fukuchi et al. 2019). The discrepancy between studies can therefore be explained most simply by their differing treatments of *H. heidelbergensis*. The results here further demonstrate that careful examination of geography and morphology below the morphospecies level are needed to develop a thorough understanding of the spatial and demographic processes that shaped the divergence and reticulation of hominin populations throughout the Pleistocene.

Support of AD relationships differs when temporal and morphological data are considered together and when morphology is considered separately (Table 1). In contrast to the combined dataset, the morphological data alone are either equivocal (*H. heidelbergensis*, *P. boisei*) or prefer a bifurcating arrangement (*H. erectus*). This may reflect the limits of morphospecies-level data. The temporal and spatial heterogeneity expected in a long-lived and widespread lineage such as *H. erectus* (Baab 2008) may simply yield too much demographic complexity to map neatly to such a coarse representation of process. Adopting a finer scale of analysis will facilitate testing of the phylogenetic cohesiveness of named Pleistocene taxa such as *H. erectus* and *H. heidelbergensis* and identify whether breaking down spatial heterogeneity can yield a more detailed understanding of the processes underlying the divergence of Pleistocene populations.

Pleistocene biogeography: The biogeographic range reconstructions suggest the presence of a geographically widespread, mid-Pleistocene ancestor to humans, Neanderthals, *H. heidelbergensis*, and *H. rhodesiensis*. Together with the ranges implied by the taxa inferred as being direct ancestors, this

suggests that Pleistocene *Homo* can be characterized by patterns in widespread dispersal, followed by gradual fragmentation into geographically-distinct subpopulations. One salient feature of the reconstructed phylogeny revealed by examining its biogeographic implications is the separation of *H. heidelbergensis* and *H. rhodesiensis* into separate geographically consistent clades. This is notable because the two taxa are often lumped together into a more geographically-widespread definition of *H. heidelbergensis*. The equivocal support for *H. erectus* budding and the phylogeographic patterns revealed in later hominins further underscore the need for examination at lower taxonomic scales than is typically undertaken. Overall, the picture of hominin evolution presented here represents an alignment of phylogenetic results with the heterogeneous geographic patterns observed in *H. erectus* and early African *H. sapiens* (Baab 2011, Hublin et al. 2017) and the complicated network of genetic interactions (Green et al. 2010, Kuhlwilm et al. 2016, Villanea and Schraiber 2019, Rogers et al. 2020) toward a deeper statistical understanding of the complexity in evolutionary and demographic patterns between hominin populations throughout the Pleistocene.

Phylogenetics and speciation processes in the fossil record: These analyses highlight the difficulty in confidently identifying fossil taxa that represent ancestral populations from species-level morphological and stratigraphic data. The result that morphological data do not provide clear support for the AD relationships identified here suggests that researchers seeking to identify AD relationships should be cautious in their interpretations while clearly representing the uncertainty and discordance across each data source. In general, I suggest that reconstructions of AD relationships that are generated from phylogenetic data should generally be treated as contingent and coarse reconstructions as opposed to detailed inferences of the processes underlying the divergence of species. Population-level phenotypic (or molecular) and spatial data are typically needed to generate a detailed understanding of speciation processes in living and fossil taxa. If satisfied with the particular scheme of OTU resolution and a coarse scale of inference, the framework here may be broadly useful to discern general patterns in phylogenetic ancestry. However, researchers using approaches that seek to delineate between competing modes of speciation should be mindful of scale. Further development of a clear understanding of the capabilities and limitations of data at multiple timescales will enable a continued expansion of the boundaries of the fossil record when speaking to fundamental questions in evolutionary biology.

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Figure

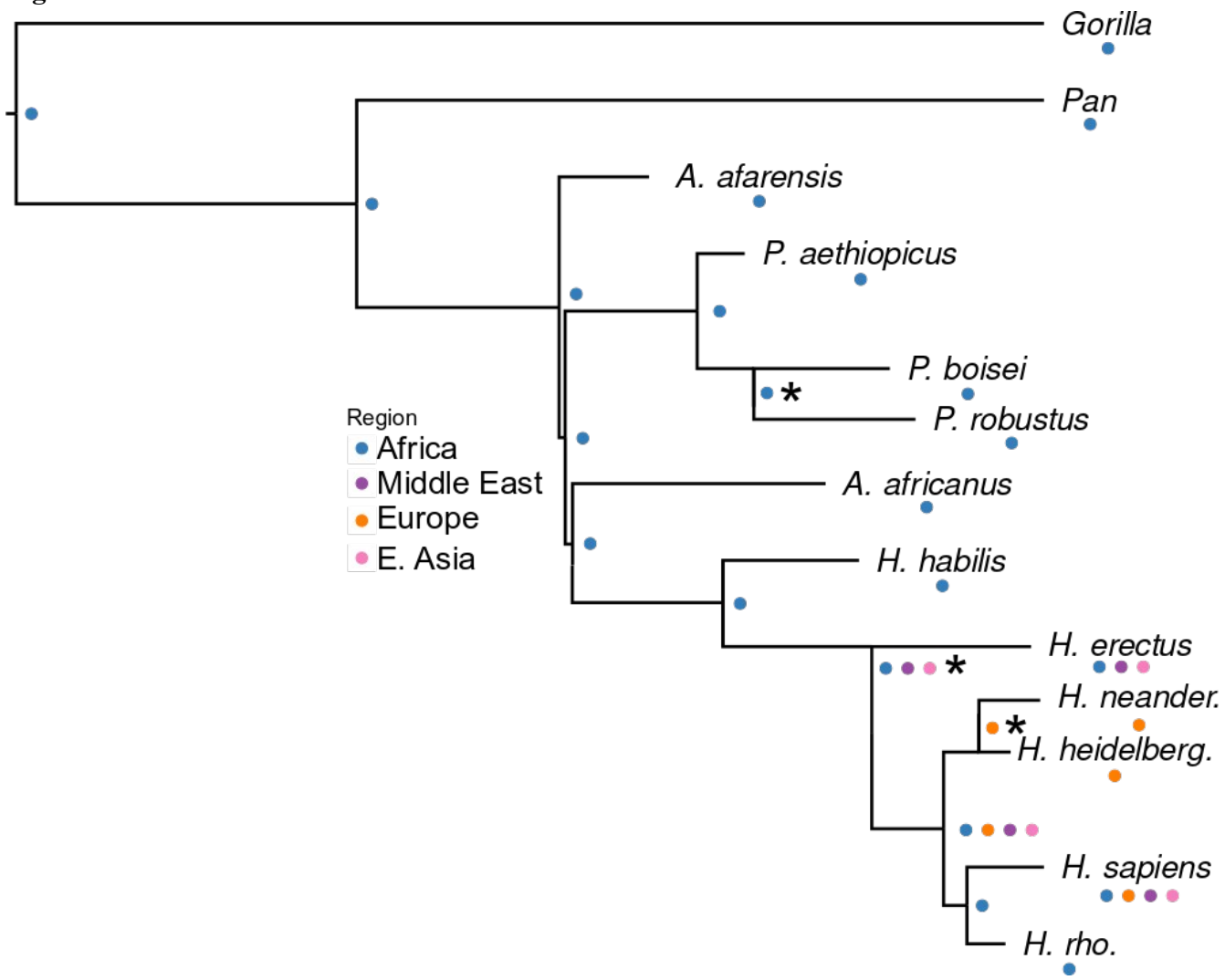

Figure 1: Best stratophylogenetic tree with geographic range reconstructions mapped to internal nodes. Asterisks denote nodes fixed in the biogeographic analysis due to their reconstruction as ancestral taxa. Geographic ranges representing tips and sampled ancestral taxa correspond only to the particular specimens contributing to the morphological data within each taxon in this study (see table 2 for full list of specimens).

Tables

OTU	Combined AIC weight	Morphological AIC weight
P. aethiopicus	0.03	0.02
P. boisei	0.75	0.51
A. afarensis	0.02	0.02
A. africanus	0.14	0.02
H. habilis	0.41	0.21
H. erectus	0.77	0.26
H. heidelbergensis	0.75	0.49
H. rhodesiensis	0.02	0.01

Table 1. Support for each candidate taxon's position as a direct ancestor on the best stratophylogenetic tree. Model support for each directly ancestral assignment is given by the AIC weight of the ancestral arrangement calculated relative to the model support for the bifurcating arrangement (see methods). The resulting support indices fall between 0-1, with values above 0.5 indicating greater support for a budding or anagenetic arrangement and below 0.5 indicating preference for a bifurcating relationship. When calculating support for each taxon, all other relationships and AD assignments in the tree were held at their ML placements. The first column reflects AIC support yielded by the combined stratigraphic and morphological data set, while the second column reflects support displayed by morphology alone.

Specimen	OTU
A.L.444-2	*Australopithecus afarensis*
Sts 5	*Au. africanus*
KNMER-406	*Paranthropus boisei*
OH 5	*P. boisei*
SK 48	*P. robustus*
WT 17000	*P. aethiopicus*
KNMER 1470	*Homo habilis*
KNMER 1813	*H. habilis*
KNMER 3733	*H. erectus*
D2700	*H. erectus*
Zhoukoudian	*H. erectus*
Steinheim	*H. heidelbergensis*
Broken Hill 1	*H. rhodesiensis*
Atapuerca 5	*H. heidelbergensis*
Gibraltar 1, Forbes' Quarry	*H. neanderthalensis*
La Chappelle-au Saints 1	*H.neanderthalensis*
La Ferrassie 1	*H. neanderthalensis*
Patagonian, Rio Negro #797	*H. sapiens*

Table 2: Specimens used in the morphometric dataset. Additional details concerning the museum storage of specimens are available from the original study (González-José et al. 2008).

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