

Relative time scales reveal multiple origins of parallel disjunct distributions of African caecilian amphibians

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Parallel patterns of distribution in different lineages suggest a common cause. Explanations in terms of a single biogeographic event often imply contemporaneous diversifications. Phylogenies with absolute time scales provide the most obvious means of testing temporal components of biogeographic hypotheses but, in their absence, the sequence of diversification events and whether any could have been contemporaneous can be tested with relative date estimates. Tests using relative time scales have been largely overlooked, but because they do not require the calibration upon which absolute time scales depend, they make a large amount of existing molecular data of use to historical biogeography and may also be helpful when calibration is possible but uncertain. We illustrate the use of relative dating by testing the hypothesis that parallel, disjunct east/west distributions in three independent lineages of African caecilians have a common cause. We demonstrate that at least two biogeographic events are implied by molecular data. Relative dating analysis reveals the potential complexity of causes of parallel distributions and cautions against inferring common cause from common spatial patterns without considering the temporal dimension.

Keywords: Africa; amphibians; Gymnophiona; historical biogeography; molecular dating

1. INTRODUCTION

Historical biogeography seeks to explain patterns of species distributions in terms of episodes of biogeographic processes such as dispersal and vicariance. Independently derived groups of species with parallel distributions suggest shared biogeographic histories. However, a 'common area relationship' might not necessarily imply common cause. An oft-overlooked requirement for inferring that a biogeographic event

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has affected multiple lineages contemporaneously is a temporal congruence (e.g. [Hunn & Upchurch 2001](#); [Donoghue & Moore 2003](#)). Given suitable calibration and robust dating methods ([Arbogast et al. 2002](#)), molecular data allow inference of absolute divergence times that can be used to test whether divergence events in multiple lineages might have occurred contemporaneously as a result of a single biogeographic event (figure 1; [Bocxlaer et al. 2006](#); [Noonan & Chippindale 2006](#)). In contrast, rejection of temporal congruence indicates that more than one biogeographic event is required to explain parallel distributions of modern lineages.

Numerous workers have noted disjunct east/west distributions of closely related taxa in the tropical forests of Africa (e.g. [Loveridge 1937](#); [Moreau 1966](#)). Phylogenetic studies have revealed three such parallel, disjunct distributions of sister-taxon pairs of African caecilians ([Nussbaum 1985](#); [Wilkinson et al. 2003](#); [Frost et al. 2006](#)). As with many other groups (and particularly those of tropical forests), caecilians have a poor fossil record ([Evans & Sigogneau-Russell 2001](#)) that provides no internal calibration points and offers limited scope for inferring a robust absolute time scale without recourse to assuming correlation between some divergence(s) and a biogeographic event that we might seek to test. However, by inferring relative rather than absolute time scales from molecular data, it is possible to test null hypotheses that explain parallel distributions as a result of contemporaneous divergences, consistent with their being caused by a single biogeographic event. Here we use this simple approach to test whether disjunct distributions of east/west African caecilians arose contemporaneously and so could have a common biogeographic cause.

2. MATERIAL AND METHODS

(a) Sampling, sequencing and phylogenetic analyses

To produce a molecular phylogeny including representatives of each of the three east/west sister-group pairs, we added new sequences for one species of *Crotaphatrema* and an additional species of *Scolecomorphus* to the alignment of concatenated partial 12S and 16S caecilian sequences of [Wilkinson et al. \(2003\)](#). Sequencing methods are as given in [Wilkinson et al. \(2003\)](#). GenBank accession number and details of voucher specimens, alignment and phylogenetic analysis are given in the electronic supplementary material.

(b) Relative dating

To assess whether the data fulfil the predictions of a molecular clock we performed a likelihood ratio test. The significance of differences in pairwise rates of molecular evolution was tested using RRTREE ([Robinson-Rechavi & Huchon 2000](#)). Molecular dating analyses were performed using r8s v. 1.70 ([Sanderson 2003](#)) MULTIDIV TIME ([Thorne & Kishino 2002](#)) and BEAST v. 1.3 ([Drummond & Rambaut 2003](#)). To evaluate temporal congruence, we fixed the root node to an arbitrary value (10) and inferred the relative timing of the divergences of the three east/west pairs. With r8s, we analysed the data using autocorrelated, penalized likelihood approaches. Bayesian analyses were performed with relaxed clocks where rates between adjacent branches were autocorrelated ([Thorne & Kishino 2002](#)) or uncorrelated with a range of prior distributions in BEAST ([Drummond et al. 2006](#)). Confidence intervals (95%) for estimated dates of divergences were used to accept or reject the hypothesis that divergences among parallel-distributed East and West African lineages are temporally congruent. For Bayesian analyses, confidence intervals were estimated directly from the sampled Markov chains. For penalized likelihood analyses, confidence intervals of estimated dates were approximated using the bootstrap ([Sanderson 2003](#)). See electronic supplementary material for further details.

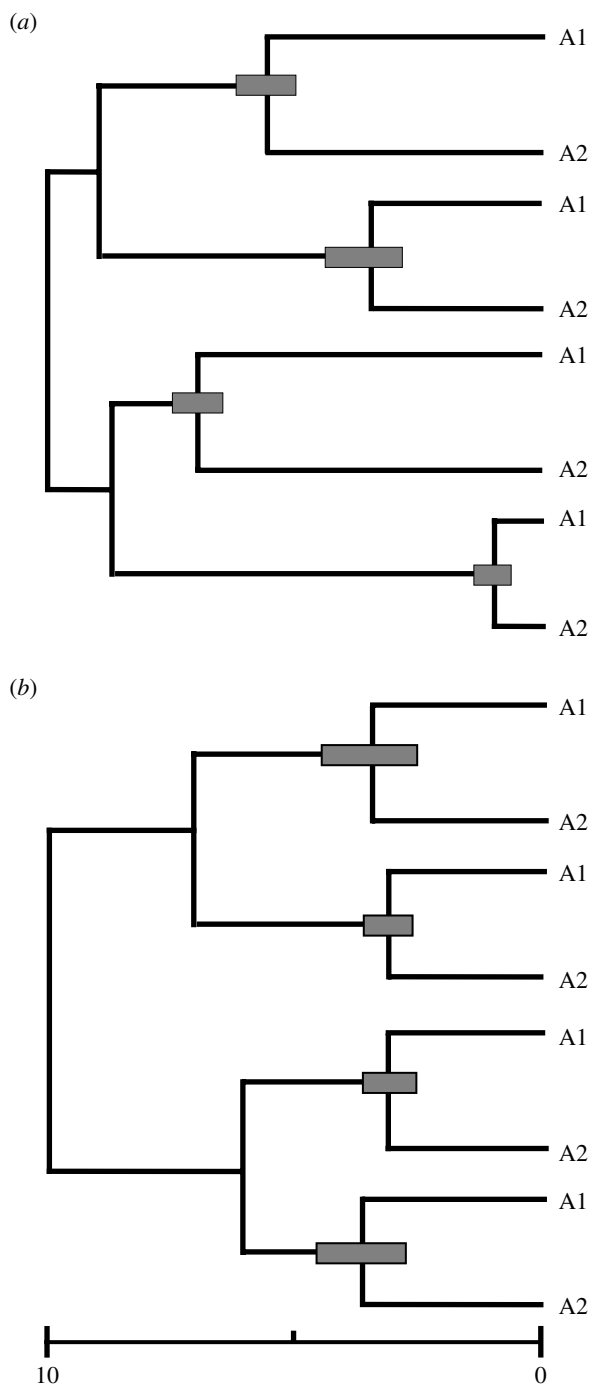


Figure 1. Timing (relative scale 0–10) of divergence events in a hypothetical phylogeny, shown as two alternative time-trees, with terminal labels showing distribution in Area 1 (A1) or Area 2 (A2). (a) null hypothesis of divergence contemporaneity rejected (b) contemporaneity not rejected.

3. RESULTS

All phylogenetic analyses yielded trees including the two east/west African sister-taxon pairs of *Schistometopum gregorii*/*Schistometopum thomense* as in Wilkinson *et al.* (2003) and *Scolecormorphus*/*Crotaphatrema* as predicted from morphology (Nussbaum 1985) and recovered from molecular analyses by Frost *et al.* (2006). The third east/west sister pair of *Boulengerula*/*Herpele* was recovered in parsimony and distance analyses with low support (as in Wilkinson *et al.* 2003), but their relationships were incompletely resolved in Bayesian and

unconstrained maximum likelihood trees. However, on the basis of high support for this pairing from the more substantial molecular data of Frost *et al.* (2006) and Roelants *et al.* (2007), we assume the sister grouping of *Herpele* + *Boulengerula* and used a correspondingly constrained maximum likelihood tree for the relative dating (see electronic supplementary material). The addition of *Scolecormorphus kirkii* provides a further test of the monophyly of *Scolecormorphus* and reveals that within this genus *Scolecormorphus vittatus* and *S. kirkii* are sister species.

The molecular clock was rejected for our data and timetrees were therefore constructed using relaxed clock approaches. There are only a few cases of significant rate variation, with no clear bias concerning African taxa (see electronic supplementary material). Although results from alternative methods differ in the precise age relative to the root age, the relative timetrees constructed using all methods agree that the split between the *Schistometopum* species pair occurred significantly later than the divergences of the two other east/west pairs (figure 2; table 1). Using the confidence intervals for discriminating between hypotheses allows us to reject the null hypothesis of contemporaneity and a single, common biogeographic cause of the parallel distributions. In contrast, confidence intervals for the estimated relative dates of divergence of *Boulengerula* and *Herpele* as well as *Crotaphatrema* and *Scolecormorphus* overlap and thus do not allow us to reject the hypothesis that parallel distributions of these two pairs of caecilian taxa are the result of a single biogeographic event.

4. DISCUSSION

(a) African biogeography

Connections between East and West African forests are thought to have become limited during the Neogene, with an overall drying of the climate (Trauth *et al.* 2005), perhaps related to geophysical activity (e.g. formation of the Rift Valley, Lovett 1993 and references therein) and leading to formidable barriers to transcontinental dispersal, and thus to gene flow, in forest-associated taxa (Lovett 1993). If, as has been suggested (e.g. Lovett 1993), these changes affected a broad range of taxa to produce the observed patterns of east/west sister-group pairs, then we would predict the temporal congruence of the divergences of these pairs with each other and with the putative geophysical and climatic causes. However, this hypothesis has yet to be tested using modern comparative methods. Although the absence of an absolute time scale prevents any test of the associations of divergences with possible extrinsic controls, relative dating allows us to reject the hypothesis that the three parallel, disjunct distributions of caecilian sister taxa in East and West Africa result from a single biogeographic event and thus provides evidence of a more complex biogeographic history (see also electronic supplementary material). Our data do not reject the hypothesis of contemporaneous divergence of *Herpele* and *Boulengerula* and of *Scolecormorphus* and *Crotaphatrema*, which merits

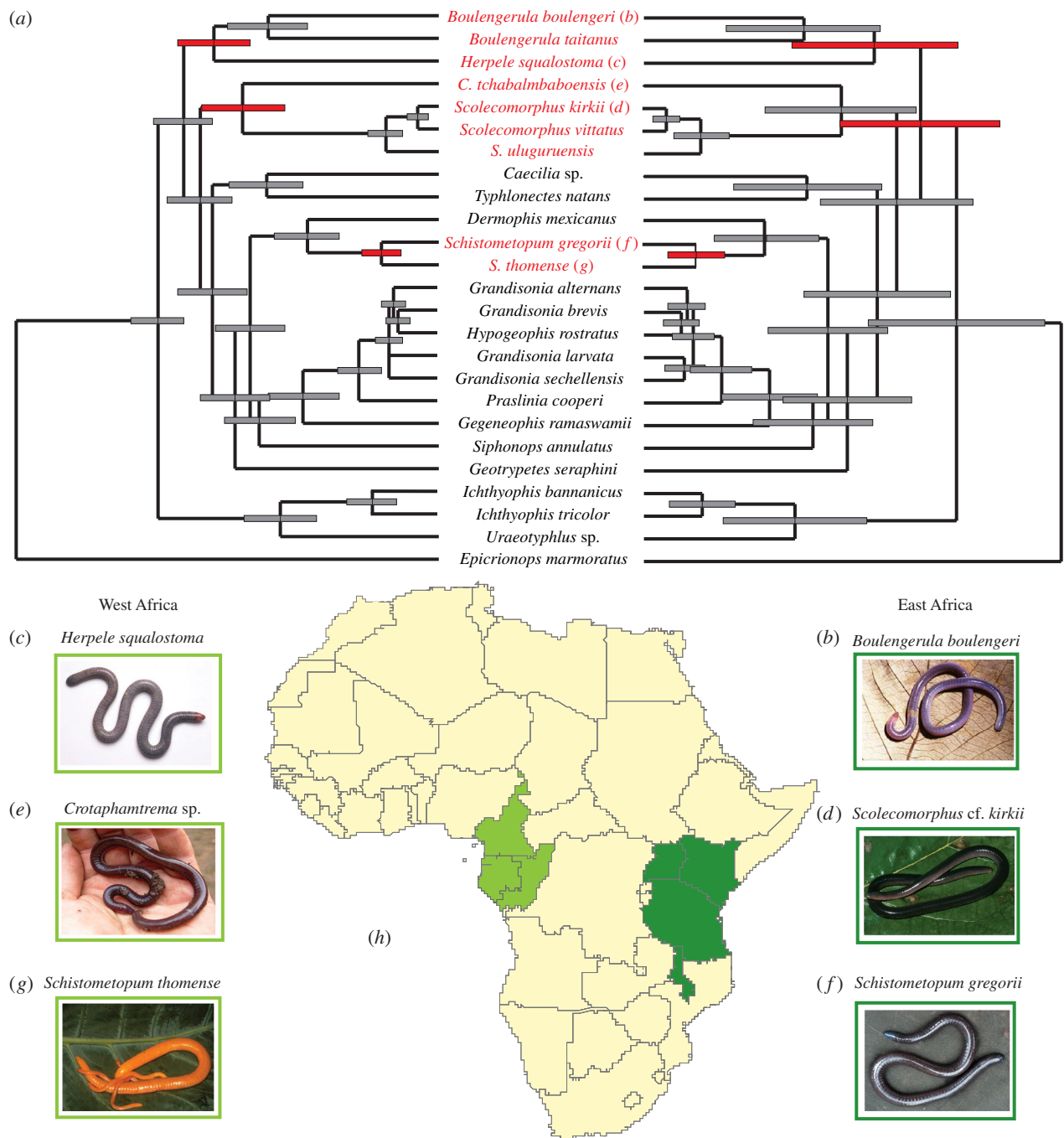


Figure 2. (a) Caecilian chronogram showing relative time using Left: penalized likelihood estimates from r8s; right: uncorrelated lognormal clock from BEAST. (b)–(g) Pictures of West (c, e, g) and East (b, d, f) African caecilians (h) Map of Africa showing the overlaid disjunct distributions of pictured East and West African caecilian genera.

Table 1. Relative estimates (scale 0–10) for the timings of parallel East/West African caecilian divergences.

	<i>Boulengerula</i> – <i>Herpele</i>	<i>Scolecomorphus</i> – <i>Crotaphatrema</i>	<i>Schistometopum thomense</i> – <i>S. gregorii</i>
r8s—penalized likelihood additive (figured in 2a)	5.23 (4.46–6.16)	4.52 (3.69–5.59)	1.28 (0.91–1.83)
r8s—penalized likelihood logpl	5.41 (4.04–6.18)	4.63 (3.35–5.50)	1.3 (0.75–1.8)
MULTIDIV TIME	8.11 (6.49–9.54)	7.10 (5.26–8.83)	2.14 (1.23–3.23)
BEAST—lognormal coalescent prior (figured in 2b)	5.50 (3.60–7.48)	4.67 (2.81–6.37)	1.26 (0.62–1.95)
BEAST—lognormal yule prior	7.76 (6.52–9.10)	6.88 (5.56–8.12)	1.97 (1.32–2.62)

further testing with additional data. Unravelling major events in tropical African biodiversity will probably require a combination of absolute and, where unavailable, relative time scales.

(b) *Relative dating*

For organisms with a poor fossil record, the prospects for obtaining accurate calibration points that do not assume biogeographic events we wish to test may be

poor. In such cases, and where secondary calibrations are also unavailable or deemed unsatisfactory (e.g. Graur & Martin 2004), testing the temporal component of biogeographic hypotheses using absolute time scales may be impossible. In the absence of a good absolute time scale, relative time estimates provide a simple approach for testing contemporaneity. As our example shows, rejection of the null hypothesis of contemporaneity can sometimes be achieved with relatively few data. The power of the approach will depend on how closely separated in time are any events that are not contemporaneous, and the breadth of the confidence intervals estimated from the available data. We are more impressed by the rejection than by the failure to reject a null hypothesis of contemporaneity, to the extent that the latter may be due to limited data.

At present, relative dating requires inclusion of all relevant taxa in a single tree and the availability of one or more common markers. Where the relevant taxa are distantly related, variation in rates of molecular evolution among lineages (Gillespie 1991) is likely to be greater, exacerbating the major difficulty for inferring divergence dates from molecular data. This, and the inability to use relative dates to test hypothesized, independently dated extrinsic causes of biogeographic patterns are major limitations that make absolute dating preferable when possible. However, the quality of absolute time scales is contingent upon the quality of calibration. Errors and/or broad confidence intervals for calibration points could mislead or otherwise reduce the power of tests of contemporaneity based on absolute dating. The immunity of relative dating to these potential problems makes it a potentially useful complementary approach, even when inference of absolute time scales is possible.

Molecular phylogenies have been used widely to test biogeographic hypotheses (e.g. Noonan & Chippindale 2006; Bocxlaer *et al.* 2006). Beyond the testing of predicted area relationships, molecular assessments of the temporal component of biogeographic hypotheses have, by and large, been restricted to attempts (sometimes *post hoc*) to align lineage divergences against dated (usually abiotic) events such as geotectonic episodes. In ignoring the potential use of relative divergence dates, we believe that molecular biogeographers have been too timid, and that there already exists a vast amount of underexploited data that can be used for biogeographic analyses even in the absence of well-calibrated, absolute time scales.

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