

Application of molecular clocks in ornithology revisited

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Molecular clocks have seen many applications in ornithology, but many applications are uncritical. In this commentary, I point out logical inconsistencies in many uses of clocks in avian molecular systematics. I call for greater rigor in application of molecular clocks – clocks should only be used when clocklike behavior has been tested and confirmed, and when appropriate calibrations are available. Authors and reviewers should insist on such rigor to assure that systematics is indeed scientific, and not just storytelling.

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Molecular clocks have been applied broadly in ornithology over recent decades, and have been key in several results of importance to understanding the evolution and biogeography of birds (Klicka and Zink 1997, Omland and Lanyon 2000, Lovette et al. 2001). Although the broader community (i.e. beyond Ornithology) has debated the utility of clocks extensively (Brochu et al. 2004, Graur and Martin 2004, Ho and Larson 2006, Rutschmann 2006), and several bird-specific commentaries have also been offered (García-Moreno 2004, Lovette 2004), application of molecular dating ‘techniques’ to avian questions is common, and acceptance of resulting dates can often be uncritical. This commentary reviews current applications of molecular clocks in ornithology to urge greater logical rigor in the use of molecular clocks in systematic ornithology, pointing out inconsistencies in a surprising number of such studies published to date. It should be noted that an entirely distinct and perhaps even more serious suite of criticisms could be leveled at this practice regarding the *calibration* of molecular clocks, but that these problems are not treated herein.

To characterize current applications of molecular clocks in ornithology, I selected 75 papers at random from the recent literature (i.e. 2000–2006) that present primary analyses of molecular (i.e. DNA sequence) variation among populations, species, or clades of birds. Papers were identified via searches in online databases (e.g. Web of Science), and through searches of contents of key journals (e.g. *Auk*, *J. Avian Biol.*, *Mol. Phyl. Evol.*). I omitted from consideration those papers that had an approach that was primarily population genetic in orientation (i.e. focusing on variation among individuals within populations). Papers reviewed, with abbreviated literature citations, are listed in Appendix I.

General steps involved in the molecular dating process should in general include (1) testing for and confirmation of clocklike evolution of genes studied in a clade, and (2) application of a specific calibration to translate genetic distances into actual dates (Fig. 1). Decisions at each of these stages have clear, logical consequences that affect both the results of the analysis *and* their believability. Nonetheless, some decisions made by some authors of such papers are not logically consistent, leading to worrisome implications and conclusions.

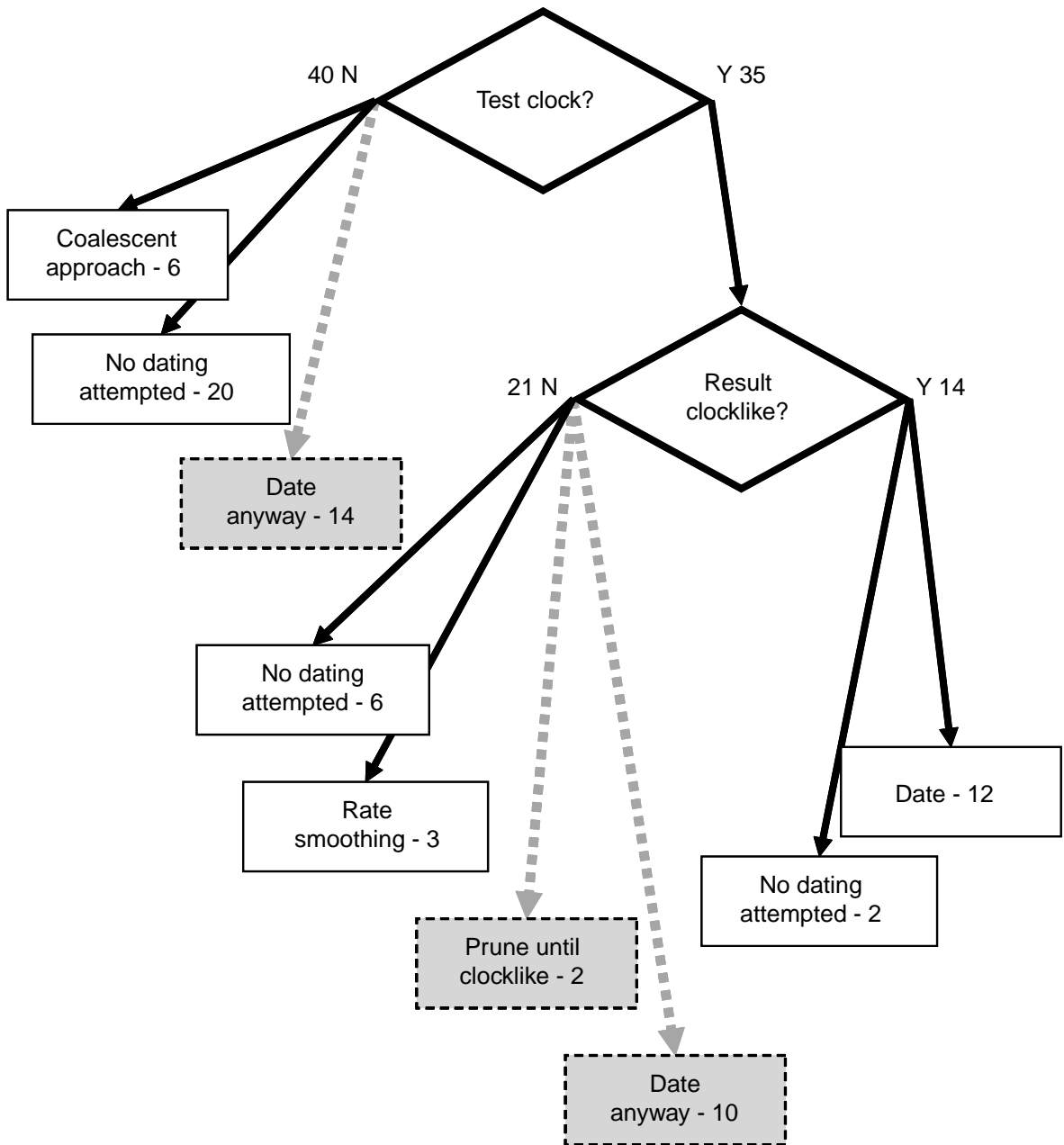


Fig. 1. Summary of testing for clocklike molecular evolution and results of tests among 75 recent (2000–2006) molecular studies of bird systematics. Boxes shown in gray represent inconsistent steps that are inappropriate as parts of a scientific process.

Testing for clocklike molecular evolution in a clade generally involves comparisons of likelihoods of trees built with and without enforcing clocklike behavior, in essence asking whether the clock-enforced tree is significantly less likely than the one without a clock enforced. In my survey, however, only 35 of the 75 studies conducted such tests; another 6 studies used coalescent approaches to dating, which do not depend on rate constancy. However, of the remaining 34 studies

(i.e. those that did not test for clocklike evolution), 14 proceeded to date splitting events anyway – this procedure is akin to using a parametric ANOVA to test for differences in central tendency without testing for normality of distributions first. Of the 35 studies that did test for clocklike evolution, 14 could not reject the null hypothesis of clocklike behavior and as such could then logically proceed to date splitting events (although 2 did not, in the end, go on to date splitting events).

More importantly, of the 21 studies for which the null hypothesis was rejected (i.e. conclude non-clocklike evolution), 10 dated splitting events using a single-rate calibration anyway; other studies either did not attempt dating (6), used rate-smoothing approaches (3), or pruned trees until the null hypothesis could not be rejected (2).

At the next step – calibrating molecular difference to time of splitting – 41 studies attempted to date splitting events using a diversity of approaches and assumptions. While several of these studies used custom calibrations to decide on how to translate genetic differences into dates, fully 27 studies used general calibrations from the literature (Fleischer et al. 1998) – the traditional “2% rule” and its descendent calibrations. Given well-documented variation in molecular evolutionary rates (see, e.g. that only 33% of studies reviewed here that tested for clocklike evolutionary rates found statistical support for clocklike evolution), the assumption that evolutionary rates in any particular group will match those of a group for which a calibration was possible [see the careful calibration developed by Fleischer and colleagues (Fleischer et al. 1998)] is tenuous at best.

Recent research has offered numerous new analytical tools for these challenges. For instance, when molecular evolutionary rates are variable, new rate-smoothing algorithms permit incorporation of this variation into dating estimates, in essence varying molecular evolutionary rates across different parts of the tree to improve the clocklike appearance of molecular evolution as reconstructed on the tree (Sanderson 2002, Welch and Bromham 2005), which could potentially alleviate some problems caused by rate variation, although these techniques are not without their controversy (Ho and Larson 2006, Rutschmann 2006). Certainly, rate smoothing is better than assumption of rate constancy, when constancy is not demonstrable.

The other approach that has been used to deal with rate variation is that of “pruning” trees until rate variation is no longer detectable statistically – here, the investigator removes fast- or slow-evolving lineages until character evolution on the tree is not distinguishable statistically from a clock-enforced tree. This alternative is odd, given that it explicitly ignores known non-clocklike evolution in lineages. If all rate variation were occurring in terminal lineages (i.e. in the “twigs” on the tree), this approach might be defensible, but internal branches nonetheless also show variable rates, making pruning untenable – it simply hides the problem. More to the point, it should be borne in mind that the statistical tests for rate variation use probability criteria to reject (or not) hypotheses – pruning inconvenient branches off of trees may reduce statistical power or remove enough of the glaring outliers, to the point that statistical significance is no longer achieved. Providing that proper tests of monophyly have been conducted (i.e. that rates are

not varying simply because taxa that are not part of a clade are included in the analysis), the underlying rate variation, nonetheless, still exists, and the problem has only been covered over, not removed.

Finally, and of particular importance for dating Pleistocene events, those investigators wishing to use molecular clocks to date events should put more attention to the implications of the temporal resolution provided by the clock. For instance, the Fleischer et al. calibration is provided with two significant digits (Fleischer et al. 1998) – if the truth were to vary from this estimate by the minimum possible (0.1%/mya), a date for 50,000 yr ago would have a possible range of 47,059–53,333 yr ago, which seems to be an acceptable narrow range. This 6000+yr range, however, includes 3 warm periods and 2 full ‘ice age’ periods (Dansgaard et al. 1993) – as such, even this minimal level of imprecision suggests that the ‘clock’ does not date events with sufficient temporal resolution to correlate lineage splitting events with climatic events. Given that climatic fluctuations occurred on extremely fine temporal scales throughout much of the Pleistocene (Siddall et al. 2006), clocks should be applied to such questions only with great care.

The point of this commentary is not that molecular clocks should never be applied to questions of bird evolutionary history and biogeography. Indeed, I have not pointed out a plethora of other concerns about molecular clock dating, such as complications with calibrations and confidence limits (Graur and Martin 2004), conflicts between short-term mutation rates *vs* long-term substitution rates (Ho and Larson 2006), and others. Rather, I simply point out the frequency with which clocks are applied inappropriately, which likely produces inaccurate and unreliable dating estimates in the ornithological literature. I aim this comment not just at the authors working on questions of molecular phylogeny and biogeography, but also to the editors and reviewers of those papers, in the hopes that they can enforce some degree of rigor when molecular clocks are applied in ornithology.

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Appendix I. List of 75 molecular systematic papers reviewed in this analysis, with short citations.

Citation

- Alström et al. (2006, *Mol. Phyl. Evol.* 38: 381–397), Arbogast et al. (2006, *Evolution* 60: 370–382), Baker et al. (2005, *Proc. Nat. Acad. Sci. USA* 102: 8257–8262), Baker et al. (2003, *Auk* 120: 848–859), Beresford et al. (2004, *Auk* 121: 23–34), Bowie et al. (2006, *Mol. Phyl. Evol.* 38: 171–188), Bowie et al. (2004, *Auk* 121: 660–681), Bridge et al. (2005, *Mol. Phyl. Evol.* 35: 459–469), Brumfield (2005, *Auk* 122: 414–432), Buehler and Baker (2005, *Condor* 107: 497–513), Burns and Barhoum (2006, *Mol. Phyl. Evol.* 38: 117–129), Cheviron et al. (2005, *Mol. Phyl. Evol.* 36: 338–357), Cibois (2003, *Auk* 120: 35–54), Cibois et al. (2002, *J. Avian Biol.* 33: 380–390), Cibois et al. (2001, *Evolution* 55: 1198–1206), Cibois et al. (2004, *Condor* 106: 837–851), Crochet et al. (2003, *Evolution* 57: 2865–2878), Crochet et al. (2002, *Auk* 119: 603–620), Dimcheff et al. (2002, *Mol. Phyl. Evol.* 24: 203–215), Dingle et al. (2006, *Auk* 123: 119–134), Drovetski and Ronquist (2003, *Journal of Biogeography* 30: 1173–1181), Drovetski et al. (2004, *J. Avian Biol.* 35: 105–110), Eberhard and Bermingham (2004, *Auk* 121: 318–332), Eberhard and Bermingham (2005, *Mol. Phyl. Evol.* 36: 288–304), Fain and Houde (2004, *Evolution* 58: 2558–2573), Fleischer et al. (2006, *Biology Letters* 10.1098/rsbl.2006.0490), Fok et al. (2002, *Proc R Soc B* 269: 1671–1679), Fuchs et al. (2006, *Mol. Phyl. Evol.* 39: 186–197), García-Moreno et al. (2006, *Mol. Phyl. Evol.* 38: 488–498), Gill et al. (2005, *Auk* 122: 121–143), Grau et al. (2005, *Mol. Phyl. Evol.* 35: 637–645), Grenier and Greenberg (2005, *Evolution* 59: 1588–1595), Griswold and Baker (2002, *Evolution* 56: 143–153), Groombridge et al. (2002, *Mol. Phyl. Evol.* 25: 267–277), Groth (2000, *Auk* 117: 787–791), Hunt et al. (2001, *Auk* 118: 35–55), Jennings and Edwards (2005, *Evolution* 59: 2033–2047), Johnson and Cicero (2004, *Evolution* 58: 1122–1130), Johnson and Clayton (2000, *Condor* 102: 864–870), Johnson et al. (2001, *Auk* 118: 874–887), Kirchman et al. (2001, *Auk* 118: 849–863), Klicka et al. (2001, *Auk* 118: 611–623), Klicka et al. (2000, *Auk* 117: 321–336), Kondo et al. (2004, *Condor* 106: 674–680), LeCroy and Barker (2006, *Am. Mus. Nov.* 3511: 1–20), Lerner and Mindell (2005, *Mol. Phyl. Evol.* 37: 327–346), Litjmaer et al. (2003, *Evolution* 57: 1411–1418), Liu et al. (2006, *Mol. Phyl. Evol.* 38: 12–19), Lovette and Bermingham (2001, *Auk* 118: 211–215), Lovette and Bermingham (2002, *Auk* 119: 695–714), Miura and Edwards (2001, *J. Avian Biol.* 32: 102–110), Moyle (2006, *Auk* 123: 487–499), Olsson et al. (2005, *Mol. Phyl. Evol.* 36: 261–276), Outlaw et al. (2003, *Auk* 120: 299–310), Pavlova et al. (2003, *Auk* 120: 744–758), Pavlova et al. (2005a, *Mol. Phyl. Evol.* 36: 669–681), Pavlova et al. (2005b, *J. Avian Biol.* 36: 322–336), Pereira and Baker (2004, *Auk* 121: 682–694), Pereira and Baker (2006, *Mol. Phyl. Evol.* 38: 499–509), Pérez-Emán (2005, *Mol. Phyl. Evol.* 37: 511–528), Pieltney et al. (2000, *Evolution* 54: 279–289), Pons et al. (2005, *Mol. Phyl. Evol.* 37: 686–699), Price and Lanyon (2002, *Auk* 119: 335–348), Price and Lanyon (2004, *Auk* 121: 224–229), Pruett et al. (2001, *Wilson Bull.* 113: 228–231), Scribner et al. (2003, *Auk* 120: 889–907), Sgariglia and Burns (2003, *Auk* 120: 346–361), Sorenson and Payne (2001, *Evolution* 55: 2550–2567), St. John et al. (2005, *Mol. Phyl. Evol.* 37: 83–90), Tavares et al. (2004, *Auk* 121: 230–242), Thomassen et al. (2005, *Mol. Phyl. Evol.* 37: 264–277), Voelker (2002, *Condor* 104: 725–739), Webb and Moore (2005, *Mol. Phyl. Evol.* 36: 233–248), Weir (2006, *Evolution* 60: 842–855), Zink (2002, *Auk* 119: 864–871).
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