Effects of Fossil Calibration Uncertainty on Divergence Dating in Ants and Bees

Seán Brady

Divergence dating has become one of the most popular applications of molecular phylogenetics over the last decade. This technique combines models of molecular evolution with information about ages of select groups within a phylogenetic tree to infer divergence times for the entire tree in the form of a chronogram. These chronograms have in turn informed many areas of evolutionary biology including historical biogeography, evolution of adaptive characters, and rates of species diversification. The information used to calibrate divergence dating studies most frequently comes in the form of fossil evidence. This paper explores some potential impacts of fossil calibration uncertainty on divergence dating results, using work from myself and collaborators on ants and bees. Among Hymenoptera in general, divergence dating studies so far have been largely restricted to these two taxa (Brady et al. 2009).

The poor preservation and vast incompleteness of the fossil record prevalent in most insect groups presents several concerns. In studies that are only able to employ a few fossil calibrations, fossils with incomplete taxonomic or temporal data may exercise a pathological influence on dating results. There are several potential avenues to assess this, but a simple and direct approach is to remove suspect calibrations and see how this changes the results. For example, Brady et al. (2006b) used divergence dating to examine the timing of eusocial evolution in sweat bees of the family Halictidae. The fossil record for halictid bees is quite poor, so only three fossil calibrations were available for this study. After conducting analyses using all three constraints, the authors removed the two recent Dominican amber calibrations and reran the analyses. The results were the same with and without this removal, indicating that eusociality evolved approximately 20–22 million years ago in three separate lineages of sweat bees.

If one is particularly concerned about the age assigned to the fossils, one could investigate whether different age assignments influence results. For example, in a divergence dating study on ants, Moreau et al. (2006) observed that there was some uncertainty of about 5 million years over the ages of three fossil strata used to provide some of their fossil minimum age calibrations. They ran separate analyses using different age assignments for these three strata, and reported that this resulted in a 28-million-year age difference for the age of extant ants. However, when Brady et al. (2006a) repeated this same procedure on a similar data set, they found a difference of only 0–2 million years. It appears that factors other

than the precise age of the three fossil strata are the cause of these discrepant age estimates.

The use of a Bayesian statistical framework allows calibrations that are more sophisticated than minimum age constraints by allowing an *a priori* distribution of times for any particular node. Determining an appropriate statistical distribution to represent fossil information can be difficult in many insect groups that lack comprehensive fossil preservation. Typically, these assigned prior distributions are at the sole discretion of the particular researcher conducting the analysis. This situation begs the obvious question concerning the potential effect of different distributions used as *a priori* calibrations on the resulting posterior ages. I explored this issue using data from a study my colleagues and I recently published (Ward et al. 2010). In this published study, we inferred divergence dates for dolichoderine ants using a 54 taxon/10 gene phylogenetic data set and seven calibrations from the fossil record. Our dating results for representative groups are provided in the first data column of Table 1. For the present article, I examined the impact of our chosen *a priori* calibration distributions by conducting additional analyses using alternative distributions, with standard deviations (s.d.) that reflect levels of variance that are much more restrictive (s.d. = 0.1) and broad (s.d. = 1.0) than we would consider plausible. Other than this alteration of *a priori* variance, the analyses were conducted under the same conditions as reported in Ward et al. (2010). Both treatments yielded results very similar to the original study (Table 1), indicating that, for this data set, our results were not sensitive to the amount of variance introduced into our prior calibrations. This finding accords with several previous studies on ants and bees that examined this topic (Brady et al. 2006b, Danforth et al. 2004, Ward and Brady 2003).

In addition to the temporal information associated with a fossil, another major source of potential uncertainty is the taxonomic assignment of fossils. Fossils are often preserved with morphological information that is highly incomplete or difficult to observe. This can cause incorrect character and taxonomic interpretations, which in turn will affect which node in a phylogenetic tree will receive the calibration information associated with the fossil. The simplest method to account for this uncertainty is to compare alternative placements to see if this influences results. For example, Schultz and Brady (2008) conducted divergence dating analyses of the fungus-growing attine ants. Because the taxonomic identity of one of the fossils used was

Table 1. Robustness of divergence dating results in dolichoderine ants from Ward et al. (2010) to fossil calibration modification. Alterations included increasing or decreasing the standard deviation (s.d.) of calibration priors and two treatments shifting stem group calibrations to the crown group. DNAPPTOFI is an acronym designating a group of genera endemic to Australia. Values are mean ages (millions of years ago) with 95% credibility limits in parentheses.

Taxon	Ward et al. 2010	s.d. = 0.1	s.d. = 1.0	Crown 1	Crown 2
Dolichoderinae	67 (61-74)	66 (63-68)	64 (56-73)	92 (73-112)	87 (75-100)
Tapinomini	57 (51-64)	58 (55-62)	54 (47-62)	78 (59-96)	76 (65-88)
Bothriomyrmecini	42 (29-54)	42 (30-55)	41 (29-53)	56 (28-82)	54 (36-72)
Dolichoderini	52 (47-57)	54 (52-57)	49 (43-55)	70 (55-88)	66 (56-78)
Leptomyrmecini	50 (42-59)	48 (43-53)	49 (40-58)	72 (54-90)	68 (54-81)
DNAPPTOFI	23 (17-30)	23 (18-29)	23 (18-29)	38 (24-50)	32 (23-41)
Azteca	14 (7-22)	14 (8-20)	14 (8-21)	34 (21-55)	27 (20-35)

doubted by some, the authors ran analyses calibrating different nodes that reflected this ambiguity. Their results showed that divergence dates differed by only 2–4 million years using these alternative calibration nodes (Schultz and Brady 2008).

A method called fossil cross-calibration can be used to provide more objective guidance on the placement of calibrations (Near and Sanderson 2004, Rutschmann et al. 2007). This technique fixes the age of each calibration separately and gauges its effect on the inferred ages for the other calibration nodes in the absence of those calibrations. Ward et al. (2010) used fossil cross-calibration to evaluate two alternative nodal placements for a calibration that was based on a fossil with dubious taxonomic interpretations. They showed that the first nodal alternative was highly inconsistent with the other fossil calibrations in the analysis, as well as with dates for Hymenoptera established from other studies. In contrast, the second nodal alternative provided plausible age estimates well within the range of the other calibrations. The authors thus selected this second node as the placement for the calibration in their final analyses.

The above examples deal with fossils with dubious taxonomic interpretation, but even given completely accurate taxonomic information for a fossil, there is some flexibility in terms of how this translates to identifying specific clades on a phylogeny to use for calibration. In general, fossils can only provide unambiguous information toward the calibration of stem groups rather than crown groups. (In a phylogeny, a stem node is one node lower toward the base than a crown node). This is because the distinctive characters that place a fossil in a given group could have evolved along the stem lineage that connects the group to its common ancestor. Wholesale application of fossils to calibrate crown groups can inflate resulting ages. For example, the dolichoderine study discussed above (Ward et al. 2010) interpreted the fossil evidence to provide stem group calibrations. To illustrate the effects of misapplication of fossil information toward crown groups, I reran these analyses by applying these calibrations to their crown groups instead. Because one stem group ("Calibration 2" of Ward et al. 2010) has fossil evidence relevant to both crown groups, which are by definition sister groups, I conducted treatments calibrating each of these crown groups in turn (Crown 1 calibrates *Liometopum* crown and Crown 2 calibrates *Tapinoma* crown). The results of these analyses clearly indicate that calibrating using crown groups substantially increases the inferred ages for taxa within Dolichoderinae, inflating the ages up to 20 million years or more (Table 1). Some published studies have indeed utilized exclusively crown group calibrations, and it is argued that the results from such work should be viewed with some skepticism.

Uncertainty in fossil calibration can be a major source of inconsistent or erroneous results in divergence dating (Ho and Phillips 2009). Here, I have briefly summarized my efforts at exploring this factor in my divergence dating studies of ants and bees. In my data sets, temporal details of the fossil record can have much less impact compared to the nodal placement of these calibrations. Because most fossils can only be placed on a phylogeny using morphological characters, this underscores the continued relevance of morphological studies to the field of molecular divergence dating. Furthermore, because other data sets both within and outside ants and bees may behave differently than the ones I have presented here, constant probing and vigilance to possible sources of error is warranted, as we continue to apply the nascent and rapidly maturing field of divergence dating to address biological questions.

References Cited

Brady, S. G., L. Larkin, and B. N. Danforth. 2009. Bees, ants, and stinging wasps (Aculeata). Pages 264-269 *in* The Timetree of Life (S. B. Hedges, and S. Kumar, eds.). Oxford University Press, New York.

Brady, S. G., T. R. Schultz, B. L. Fisher, and P. S. Ward. 2006a. Evaluating alternative hypotheses for the early evolution and diversification of ants. Proceedings of the National Academy of Sciences of the United States of America 103: 18172-18177.

Brady, S. G., S. Sipes, A. Pearson, and B. N. Danforth. 2006b. Recent and simultaneous origins of eusociality in halictid bees. Proc. R. Soc. B 273: 1643-1649.

Danforth, B. N., S. G. Brady, S. D. Sipes, and A. Pearson. 2004. Single-copy nuclear genes recover Cretaceous-age divergences in bees. Syst. Biol. 53: 309-326.

Ho, S. Y. W., and M. J. Phillips. 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. Syst. Biol. 58: 367-380.

Moreau, C. S., C. D. Bell, R. Vila, S. B. Archibald, and N. E. Pierce. 2006. Phylogeny of the ants: Diversification in the age of angiosperms. Science 312: 101-104.

Near, T. J., and M. J. Sanderson. 2004. Assessing the quality of molecular divergence time estimates by fossil calibrations and fossil-based model selection. Phil. Trans. R. Soc. B 359: 1477-1483.

Rutschmann, F., T. Eriksson, K. A. Salim, and E. Conti. 2007. Assessing calibration uncertainty in molecular dating: The assignment of fossils to alternative calibration points. Syst. Biol. 56: 591-608.

Schultz, T. R., and S. G. Brady. 2008. Major evolutionary transitions in ant agriculture. Proc. Natl. Acad. Sci. USA. 105: 5435-5440.

Ward, P. S., and S. G. Brady. 2003. Phylogeny and biogeography of the ant subfamily Myrmeciinae (Hymenoptera: Formicidae). Invertebr. Syst. 17: 361-386.

Ward, P. S., S. G. Brady, B. L. Fisher, and T. R. Schultz. 2010. Phylogeny and biogeography of dolichoderine ants: Effects of data partitioning and relict taxa on historical inference. Syst. Biol. 59: 342-362.

Seán Brady is a Research Entomologist and Curator of Hymenoptera at the National Museum of Natural History, Smithsonian Institution. His research centers on the phylogenetics and systematics of Hymenoptera, especially bees and ants, with particular focus on social evolution, coevolutionary dynamics, molecular evolution, and historical biogeography.