Ancient DNA from Amber Fossil Bees?
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Amber fossils of insects provide excellent preservation of several tissues (e.g., Poinar and Hess 1982), and two groups have published claims of amplification of ribosomal RNA gene sequences from four amber fossil insect specimens (Cano et al. 1992, 1993; DeSalle et al. 1992; DeSalle, Barcia, and Wray 1993; DeSalle 1994). Unlike amplifications of ancient DNA from thousand-year-old mammalian specimens (e.g., Taylor 1996), no independent replications and no extensions of this work have been reported. We initially attempted to extend this work to transposable elements of the mariner and Tcl families likely to occur in insect genomes (Robertson 1993; Avancini, Walden, and Robertson 1996). These transposons commonly occur in high copy numbers (Robertson 1993; Avancini, Walden, and Robertson 1996). These transposons would be particularly useful, because it is otherwise difficult to estimate the ages of transposons in host genomes. We focused on the Dominican amber fossil stingless bee Proplebeia dominicana, and began by obtaining partial sequences of mariner (Robertson et al. 1997) and Tcl (Avancini, Walden, and Robertson 1996) family transposons from four extant relatives (see below). PCR primers designed to conserved sequences of elements shared between several of these extant relatives were thought likely to be able to amplify elements from P. dominicana; however, we were unable to obtain amplifications from ancient DNA extractions provided by R. J. Cano, nor was his laboratory able to obtain amplifications from fresh extractions (R. J. Cano and D. Da Silva, personal communication). We therefore attempted to replicate the claimed amplifications of 18S rRNA gene sequences from a single specimen of P. dominicana (Cano et al. 1992).

We initially amplified, cloned, and sequenced in both directions the first third of the 18S rRNA gene (630 bp) from DNA extractions of four extant relatives of the fossil meliponine bee, Melipona panamica, Nogueirapis mirandula, Plebeia frontalis, and P. jatiformis (Michener 1990) (kindly supplied by R. J. Cano), and the honey bee Apis mellifera for comparison. The consensus sequences from multiple clones from each species revealed extensive problems with the 232-bp sequences previously reported for P. frontalis and P. jatiformis (Cano et al. 1992). As shown in figure 1, the published sequences differ from ours at 22 positions and have several small deletions totaling 9 bp. Our sequences agree well with that of Apis mellifera (fig. 1) and other hymenopteran sequences (e.g., Carmean, Kimsey, and Berbee 1992), so we conclude that severe errors occurred in the sequencing of these genes by Cano et al. (1992). The reported sequence for the Dominican amber fossil specimen of P. dominicana has similar errors (fig. 1), so we attempted to replicate this work.

We used nine amber fossil specimens of P. dominicana, purchased from The Amber Lady (http://goldray.com/amberlady/). Although the exact mine from which these fossils originated is unclear, they were confirmed by C. D. Michener (personal communication) to be P. dominicana, and all Dominican amber derives from the same 15–20-Myr-old deposit (Iturralde-Vincent and MacPhee 1996). DNA extractions were performed by previous methods (Cano et al. 1992, 1993; DeSalle et al. 1992; DeSalle, Barcia, and Wray 1993), as well as grinding of the excised bee material on dry ice, followed by proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation; or extraction using Chelex (Walsh, Metzger, and Higuchi 1991). Control extractions were performed on surrounding amber material from each specimen. These extractions were performed in a different laboratory on another floor of our building or in an adjacent building using equipment and reagents not previously exposed to any insect DNA extractions or 18S rRNA amplifications. Multiple PCR amplifications were attempted from each extraction using primers to positions 1–20 (20F) and 145–163 (145R) of the 18S rRNA gene. This 160-bp region is immediately 5′ of that previously amplified (Cano et al. 1992), and the primers were designed to conserved regions present in most animals. No products were observed on agarose gels stained with ethidium bromide after 40 amplification cycles using standard PCR conditions (Robertson 1993) supplemented to 4 μM/ml with bovine serum albumin (Pääbo 1990).

To further explore the possibility of obtaining ancient DNA, we greatly enhanced the sensitivity of the assay by performing nested amplifications starting with a small sample of the first amplification from four specimens and associated control extractions. The nested primers, named 23F and 134R, partially nested primers to positions 1–20 (20F) and 145–163 (145R) of the 18S rRNA gene. This 160-bp region is immediately 5′ of that previously amplified (Cano et al. 1992), and the primers were designed to conserved regions present in most animals. No products were observed on agarose gels stained with ethidium bromide after 40 amplification cycles using standard PCR conditions (Robertson 1993) supplemented to 4 μM/ml with bovine serum albumin (Pääbo 1990).

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Key words: amber fossil, ancient DNA, Proplebeia dominicana, meliponine bee, 18S ribosomal RNA, polymerase chain reaction (PCR).

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other three appear to derive from beetle, human, and fish DNA (fig. 2). Because neither we nor others in our building have amplified 18S rRNA sequences from beetles or humans, these contaminants must have originated from DNA molecules in the environment. We therefore consider it most likely that the meliponine bee sequence originated not from the amber fossil bee extraction, but rather from our earlier work above and apparently from *N. mirandula*, because they share a T-A transversion at position 75 (fig. 2).

Our failure to replicate the claim of ancient DNA in these amber fossil bees (Cano et al. 1992) is robust because we performed multiple experiments on multiple specimens, attempted to amplify a shorter region, and eventually used highly sensitive nested amplifications without convincing success. Recently, another group has similarly reported failure to replicate amplifications of 18S rRNA sequences from 10 specimens of *P. dominicana*, as well as two other Dominican amber specimens and three younger copal specimens from Africa, despite the fact that they also used nested amplifications (Austin et al. 1997). These failures indicate that amplification of DNA from amber fossil insects is not as facile as previously claimed, with simple amplification from single specimens being reported in each study. In addition, they bring other claims of amplifications from amber fossil insect specimens into question. The claim of DNA amplification from a 120–135-Myr-old fossil weevil in Lethanese amber (Cano et al. 1993) cannot be replicated because it was a unique specimen (Poinar and Poinar 1994) and, in light of our results, is extremely questionable. The claims of amplification from a few specimens of Dominican amber fossil termites (DeSalle et al. 1992; DeSalle, Barcia, and Wray 1993) and wood gnats (DeSalle 1994) should be treated with caution until independently replicated. It seems likely that despite the remarkable preservative powers of amber and chemical evidence of preservation of DNA (Poinar et al. 1996), intact DNA of substantial length amenable to amplification is no longer present in these fossils (Lindahl 1993a, b). Following failure to replicate claimed DNA amplification from 17-Myr-old fossil plant DNA (e.g., Sidow, Wilson, and Pääbo 1991), and in light of the alternative explanation for DNA amplification from 80-Myr-old fossil dinosaur bones (e.g., Collura and Stewart 1995), it appears that prospects for obtaining ancient DNA from million-year-old fossils are slim.

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**Fig. 1.—** Alignments of 18S rRNA gene sequences from extant bees and the sequenced *Propyleia dominicana* (Cano et al. 1992; last three sequences). The position numbers are for the honey bee *Apis mellifera* sequence. Length (#) and sequence (*) differences between our sequences and the published extant bee sequences are indicated. Our sequences are available from GenBank as accession numbers U80834-U89838.
LITERATURE CITED


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