Problems of reproducibility—does geologically ancient DNA survive in amber-preserved insects?

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SUMMARY

Apparently ancient DNA has been reported from amber-preserved insects many millions of years old. Rigorous attempts to reproduce these DNA sequences from amber- and copal-preserved bees and flies have failed to detect any authentic ancient insect DNA. Lack of reproducibility suggests that DNA does not survive over millions of years even in amber, the most promising of fossil environments.

1. INTRODUCTION

Several recent reports claim to have isolated geologically ancient DNA from fossil dinosaur bones (Woodward *et al*. 1994), plant leaves (Golenberg *et al*. 1990; Soltis *et al*. 1992) and amber-preserved insect and plant inclusions (Cano *et al*. 1992*a*, *b*, 1993; DeSalle *et al*. 1992, 1993; Poinar *et al*. 1993; DeSalle 1994) many millions of years old. Although apparently authentic ancient DNA was recovered, empirical and theoretical evidence has cast doubt on these results (Pääbo & Wilson 1991; Sidow *et al*. 1991; Lindahl 1993, 1996; Soltis *et al*. 1995; Zischler *et al*. 1995; Poinar *et al*. 1996). Whether geologically ancient DNA exists or not remains controversial, largely because previous claims have not been verified by independent replication, a primary criterion of authenticity (Pääbo *et al*. 1989; Lindahl 1993; Handt *et al*. 1994).

Amber, fossilized tree resin, appears to hold some promise for preserving geologically ancient DNA because of the exceptional morphological (Grimaldi *et al*. 1994) and biochemical (Poinar *et al*. 1996) preservation seen in many entombed animals and plants. The amber resins are thought to dehydrate the tissues of trapped organisms and inhibit microbial degradation (Henwood 1993). However, amber may not protect DNA entirely from decay as it is permeable to gases, some liquids and has prolonged contact with seawater during its formation (Beck 1988; Hopfenberg *et al*. 1988; Poinar 1992). Several reports detail ancient DNA sequences extracted from a variety of amber-preserved organisms: stingless bees *Proplebeia dominicana* (Cano *et al*. 1992*b*), termites *Mastotermes electrodominicus* (DeSalle *et al*. 1992, 1993), a beetle *Libanorhinus succinus* (Cano *et al*. 1993), wood gnats *Valeseguya disjuncta* (DeSalle 1994) and a plant *Hymenaea protera* (Poinar *et al*. 1993). However, none has been independently replicated on the same species, and claims of reproducibility (Grimaldi *et al*. 1994; Poinar 1994) remain anecdotal.

Here we describe attempts to reproduce the isolation of ancient DNA from the stingless bee, *Proplebeia dominicana*, from Oligocene Dominican amber, the first amber-preserved organism from which claims of ancient DNA extraction and amplification were made (Cano *et al*. 1992*a*, *b*). In addition, we have attempted to isolate ancient DNA from two other species of insects of different body size, age and origin, a larger species of bee, *Trigona gribodoi*, from Quaternary East African copal and smaller specimens of two genera, *Megaselia* and *Puliciphora*, of scuttle flies (Diptera: Phoridae) from Oligocene Dominican amber, to survey the potential for DNA preservation in fossilized tree resins.

2. MATERIALS AND METHODS

(*a*) *Sample preparation and DNA extraction*

All specimens were carefully examined to ensure their authenticity, that they contained tissue remains, were entirely encased within the resin, were not crossed by any fractures or cracks, and only contained a single insect and no other inclusions (e.g. air bubbles, plant or soil matter). All manipulation of specimens, extraction of DNA and PCR set-up were conducted in a laminar flow hood kept under continuous short-wave (254 nm) UV irradiation when not in use (Ou *et al*. 1991), in a physically remote and dedicated laboratory never previously used for any DNA work. Additional precautions to eliminate contamination by contemporary DNA included regular decontamination of all surfaces and equipment (including pipettes) with a 10% sodium hypochlorite solution (Prince & Andrus 1992); aliquoting and irradiation of tubes and non-UV-sensitive solutions with both short- (254 nm) and long-wave (402 nm) UV light (Ou *et al*. 1991); and the use of dedicated protective clothing, equipment and reagents.

Amber and copal pieces were surface-sterilized as previously described (Cano *et al*. 1992*a*), or by soaking in 5% sodium hypochlorite for 10 min, rinsing twice in sterile water (Sigma, St Louis, MO), once in 70% ethanol and finally by flaming. The insect inclusions were exposed using a thermal-fracture method (Cano *et al*. 1992*a*) or by cutting away the

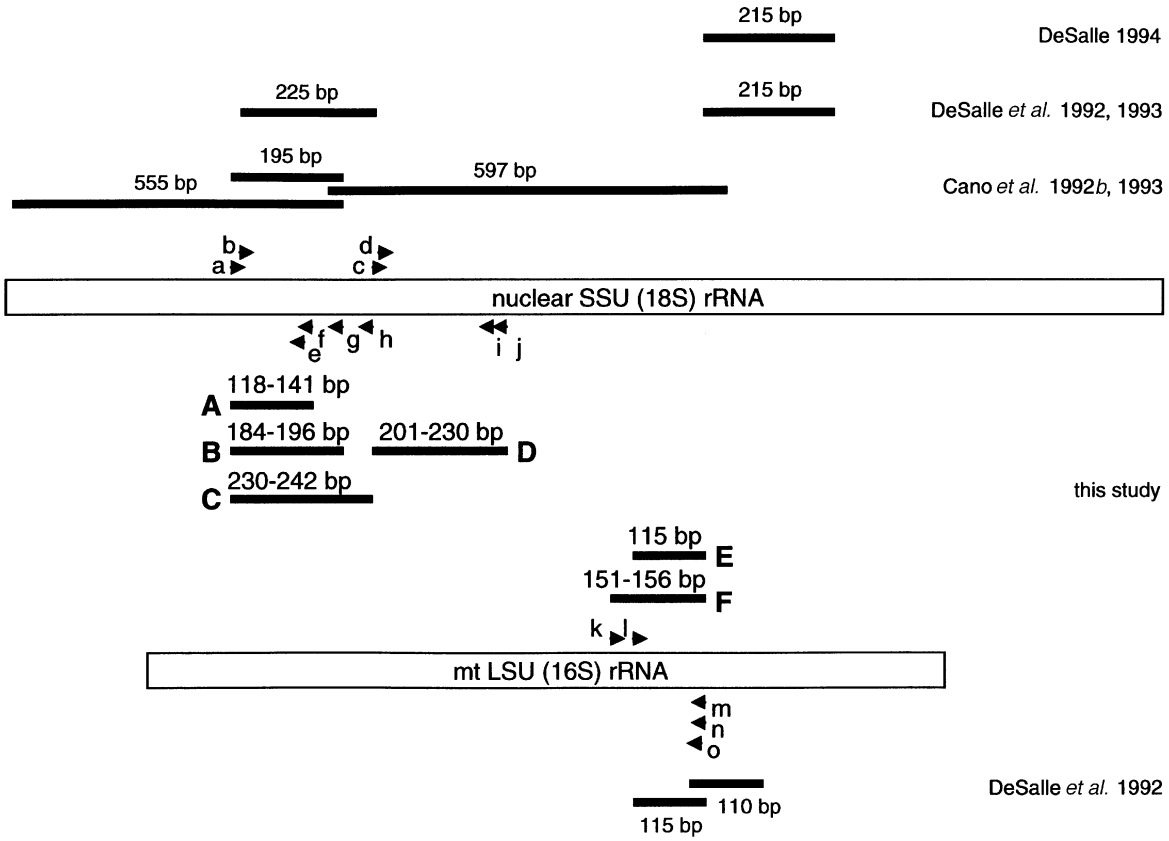

Figure 1. Location and size of PCR targets on the nuclear SSU and mitochondrial LSU rRNA genes from amber- and copal-preserved insects. PCR fragment sizes include primer sequences and are based on *Drosophila melanogaster* (nuclear) and *Apis mellifera* (mitochondrial) sequences. Upper case letters identify each target region amplified either in single or secondary/nested PCR assays. Lower case letters identify primers listed in §2. The location and size of PCR fragments amplified in previous studies of amber-preserved insects are shown for comparison. Fragment sizes are given as stated in the respective papers with the exception of the mitochondrial PCR targets of DeSalle *et al.* (1992), which were quoted as being 150 bp each.

surrounding resin (DeSalle *et al.* 1992), and tissue was removed using sterile 21 gauge needles prewetted in extraction buffer or by repeatedly pipetting 10–15 µl of extraction buffer into the body cavity and subsequently removing the buffer/tissue suspension. DNA was extracted using (i) the silica method of Höss & Pääbo (1993); (ii) the GeneClean for ancient DNA Kit (BIO 101, La Jolla, CA) as per the manufacturer's instructions; (iii) a method in which tissue was suspended in 200 µl of extraction buffer (50 mM Tris-HCl, 25 mM NaCl, 25 mM EDTA pH 8.0, 1% SDS, 0.2 mg ml^{-1} proteinase K) and incubated overnight at 55 °C with constant shaking. Three volumes of 6 M NaI (GeneClean II, BIO 101. La Jolla, CA) and 5 µl of silica suspension (Boom *et al.* 1990) were added and mixed at room temperature for 1 h. The silica was pelleted by centrifugation at 13000 rpm for 15 s, washed three times with 500 µl of ice-cold NewWash (GeneClean II) and dried at 56 °C for 15 min. DNA was eluted from the silica twice with 30 µl of sterile distilled water by incubation at 56 °C for 10 min; (iv) the Chelex method (Walsh *et al.* 1991; Cano *et al.* 1992*b*). The Chelex was made in 10 mM Tris-HCl (pH 8.0); or (v) a proteinase K/phenol:chloroform method (Cooper 1994). All five of these methods have been used with apparent success on ancient specimens, including amber-preserved organisms and a range of Pleistocene- and Holocene-age material (Cano *et al.* 1992*b*, 1993; DeSalle *et al.* 1992; Cano & Poinar 1993; Höss & Pääbo 1993; Poinar *et al.* 1993; Cooper 1994; Hagelberg *et al.* 1994; Höss *et al.* 1994, 1996; Taylor 1996). Duplicate extraction controls (no tissue added) as well as extractions from pieces of amber or copal surrounding, but not in contact with the insect specimen, were run in parallel with all insect tissue extractions.

(*b*) *PCR amplification*

Aliquots (5 µl) of extracted DNA, or 1:10 and 1:100 dilutions, were subjected to PCR amplification using primers targeting short fragments (115–242 bp) of the nuclear small subunit (SSU, 18S) and mitochondrial large subunit (LSU, 16S) rRNA genes in single (PCR targets B and C), secondary (using the same primers, PCR targets E and F) and nested (using internal primers, PCR targets A and D) PCR amplification assays (figure 1). The same regions from both of these genes have been amplified previously from four species of amber-preserved insect (figure 1). PCR primers for the nuclear SSU rRNA were:

(a) NS19, 5′-CCGGAGAAGGAGCCTGAGAAAC-3′ (386–407) (Gargas & Taylor 1992);

(b) 18Sai, 5′-CCTGAGAAACGGCTACCACATC-3′ (398–419) (DeSalle *et al.* 1992);

(c) 18Sa10a, 5′-AAGCTCGTAGTTGAATCTGT-3′ (630–649);

(d) 18Sa10b, 5′-GTAGTTGAATCTGTGTSYCAC-3′ (636–656);

(e) 18Sb9a, 5′-ATTACGGGGCCTCGGATGAGT-3′ (515–495);

(f) 18Sb9, 5′-TACTCATTCCGATTACGGGGC-3′ (526–506);

(g) NS2, 5′-GGCTGCTGGCACCAGACTTGC-3′ (581–561) (White *et al.* 1990);

(h) 18Sbi5.0, 5′-TAACCGCAACAACTTTAAT-3′ (627–609) (DeSalle *et al*. 1992);
(i) 18Sb11b, 5′-GTTCAAAGTAAACGTACCGGC-3′ (836–816);
(j) 18Sb11, 5′-GCCTGCTTTAAGCACTCTAATTTG-3′ (859–836); and for the mitochondrial LSU rRNA were:
(k) 16SL1, 5′-GACTGTACAAAGGTAGCATAA-3′ (13858–13838) (H. Poinar, personal communication);
(l) 16S1, 5′-AAGGCTGGAATGAATGGTTGG-3′ (13819–13799) (DeSalle *et al*. 1992);
(m) 16SH1b, 5′-CTATAGGGTCTTATCGTCCCAT-3′ (13708–13729);
(n) 16SH1, 5′-AAATTCTATAGGGTCTTATCGTC-3′ (13703–13725) (H. Poinar, personal communication);
(o) 16S2 5′-GATTTATAGGGTCTTCTCGTC-3′ (13705–13725) (DeSalle *et al*. 1992).

Lower case letters in parentheses identify the primers in figure 1. Numbers in parentheses refer to the position of the 5′ and 3′ nucleotides of each primer in the *Drosophila melanogaster* SSU rRNA nuclear gene and *Apis mellifera* mtDNA. Primer sequences were designed by one of us (J.J.A., see below) unless otherwise indicated. Primers a, b, g and h, and k, n and o are 'universal' for eukaryote nuclear and animal mitochondrial DNA, respectively. The remaining primers exclude fungal (e and f), or vertebrate and fungal (c, d, i, j, l and m) DNA as possible templates for PCR. Initial PCR attempts on the nuclear SSU rRNA gene used the primers a, b, g and h from previous studies of DNA from amber-preserved insects (Cano *et al*. 1992*b*, 1993; DeSalle *et al*. 1992, 1993). However, because of the wide phylogenetic range over which these primers are effective and the short length of PCR target, contamination of DNA extraction or PCR reagents became an almost routine problem despite rigorous anticontamination measures. This forced the design of primers c, d, e, f, i and j, based on an alignment of 18 insect, eight invertebrate, six vertebrate and five fungal sequences, to exclude these obvious sources of contamination. The mitochondrial primer m was designed to exclude human DNA.

PCR amplifications were done in 50 μl volumes with 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM of each primer and 1 unit of *Taq* DNA polymerase (Perkin Elmer or Promega), using buffers supplied by the respective manufacturers, with or without the addition of up to 200 μg ml^{-1} of bovine serum albumin and the use of a wax-mediated hot start. Reactions were carried out in 0.2 ml tubes in a PE9600 thermal cycler (Perkin Elmer). Primary amplifications involved denaturation at 94 °C for 60–120 s, followed by 40 cycles of denaturation at 94 °C for 40 s, annealing at 48–55 °C for 60 s and extension at 72 °C for 90–120 s, with a final extension of 5 or 10 min at 72 °C. Secondary amplifications were carried out using 2 μl of the primary reaction for 30 cycles with an annealing temperature of 55 °C or 60 °C using the same or internal (nested) primers. PCR conditions were routinely tested on positive control DNA and yielded PCR product of the correct size from approximately ten starting template molecules. PCR negative controls (no template DNA) were included in every amplification attempt, in addition to amplifications from all extraction controls. Amplification products were assessed by electrophoresis on low-melt agarose gels and PCR product of the expected size was purified and concentrated using Qiaex II (Qiagen).

(*c*) *DNA sequencing*

PCR products were cloned using the pGEM-T vector (Promega) and Epicurian coli XLI-blue MRF′ super competent cells (Stratagene). Positive colonies were checked for correctly sized inserts using a PCR assay to amplify the region between the vector's SP6 and T7 promoter sites. PCR products of the correct size from this assay were used as templates for manual cycle sequencing, as previously described (Embley 1991).

(*d*) *Phylogenetic analyses*

Sequences were aligned to those from human (*Homo sapiens*, X03205), frog (*Xenopus laevis*, K01373), dipteran fly (*Drosophila melanogaster*, M21017/M29800), hymenopterans (*Caenochrysis doriae*, L10179, *Epyris sepulchralis*, L10180, *Priocnemus oregana*, L10181), aphid (*Acyrthosiphon pisum*, X62623/S75504), beetle (*Tenebrio molitor*, X07801), zygomycete (*Gigaspora margarita*, X58726), ascomycete (*Saccharomyces cerevisiae*, M27607/J01353, *Aspergillus fumigatus*, M55626) and basidiomycete (*Athelia bombacina*, M55638). Phylogenetic trees were reconstructed using the neighbour-joining algorithm with the Kimura 2-P model in the PHYLIP package (Felsenstein 1993).

(*e*) *Authentication of DNA sequences*

We applied the following criteria to authenticate any amplified DNA sequences recovered from these insects (Pääbo *et al*. 1989; Handt *et al*. 1994).

1. Negative controls. Extraction blanks and PCR negative controls should be devoid of specific PCR amplification product.
2. Reproducibility. Amplified sequences should be consistently and reproducibly obtained from the same extract(s) from any one specimen and from different specimens of the same species.
3. Phylogenetic consistency. Amplified sequences should show unambiguous affinities with other insect sequences in a phylogenetic analysis, and therefore should be phylogenetically consistent with their supposed insect origin.

3. RESULTS

DNA extraction was attempted from 15 specimens of fossil insect, representing three species and body sizes, and two different localities, fossil resins and ages (table 1). A total of 156 PCR attempts were made on extracts from insect tissue and a further 334 PCR reactions on control extracts from the resin surrounding each insect, extraction blanks and PCR negative controls. In almost all cases, no PCR product of the expected size was detected, even after secondary amplifications, and no specific PCR product was ever detected using insect-specific primers for the target regions D, E and F. Twenty amplifications from insect tissue, all targeting the nuclear SSU rRNA gene, gave PCR product of the expected size. However, in 13 cases extraction blanks and/or PCR negative controls also yielded PCR product, and therefore the amplified sequences from insect extracts cannot be excluded as contaminants derived during the DNA extraction or PCR set-up. The remaining seven amplification products from four amber- and copal-preserved bees for PCR targets A, B and C were all amplified in experiments where both the extraction blanks and PCR negative controls were clean (table 1). Two of these products represent amplifications from separate extracts from the same specimen (sample 13, *T. gribodoi* 1). DNA was also amplified from extracts of the resin surrounding three of these bees (table 1).

These PCR products were cloned, and between three

Table 1. *DNA extraction and PCR attempts on amber- and copal-preserved insects*

(Results are expressed as the number of successful PCR amplifications from insect extractions where no amplifications were seen from extraction blanks or PCR negative controls/the total number of PCR attempts on extractions from insect tissue for that specimen and PCR target combination. Asterisks indicate PCR product amplified from extracts of amber or copal in addition to the PCR product obtained from insect tissue.)

sample	DNA extraction method†	PCR target‡ nuclear SSU rRNA				mt LSU rRNA	
		A	B	C	D	E	F
amber, Dominican Republic, 25–35 Ma							
1. *P. dominicana* 1 (BMNH Pal. PI II.267)	i	1*/2	0/3	0/3	0/3	0/2	—
2. *P. dominicana* 2 (BMNH Pal. PI II.268)	i	1*/3	0/3	0/3	0/3	0/3	—
3. *P. dominicana* 3 (BMNH Pal. PI II.638)	ii	0/2	—	—	0/2	0/2	—
4. *P. dominicana* 4 (G. Poinar Collection)	iv	0/1	—	—	0/8	0/1	—
5. *P. dominicana* 5 (BMNH Pal. PI II.269)	v	0/3	—	—	0/3	0/4	—
6. *P. dominicana* 6 (G. Poinar Collection)	v	0/1	—	—	0/1	0/1	—
7. *P. dominicana* 7 (G. Poinar Collection)	iv	0/3	—	—	0/3	0/3	—
8. *P. dominicana* 8 (G. Poinar Collection)	iv	0/3	—	—	0/3	0/3	—
9. *P. dominicana* 9 (G. Poinar Collection)	iv	0/2	—	—	0/2	0/2	—
10. *P. dominicana* 10 (G. Poinar Collection)	ii	0/2	—	—	0/2	0/2	—
11. *Megaselia* sp. (BMNH Pal. PI II.216)	ii	0/4	—	—	0/4	0/2	0/4
12. *Puliciphora* sp. (BMNH Pal. PI II.215)	v	0/1	—	—	0/1	0/1	0/2
copal, East Africa, < 2 Ma							
13. *T. gribodoi* 1 (BMNH Pal. In.38991)	iii	0/2	2*/4	0/2	0/6	0/2	—
14. *T. gribodoi* 2 (BMNH Pal. In.38989)	iii	1/3	1/6	1/3	0/3	0/9	—
15. *T. gribodoi* 3 (BMNH Pal. In.38983)	v	0/3	—	—	0/3	0/4	—

† See §2.
‡ See figure 1.
BMNH Pal.: British Museum (Natural History) Palaeontology Department.

and 14 clones were sequenced for each. There were 31 unique sequences among the 92 clones (figure 2). Twenty-eight of these sequences were unique to a single sample/PCR target combination and only three were common to two or more samples. Two sequences for PCR target A from *P. dominicana* 1, despite being the correct size, are composed entirely of tandem repeats of the forward or reverse primers b and f. The remaining 29 cloned sequences do not meet our second criterion of authenticity. Amplified sequences were not reproducible when multiple PCR attempts were made on extracts from the same bee, nor were the sequences consistent or identical between amplifications from the same or different specimens. The amplified sequences overlap with one of the fragments amplified from *P. dominicana* by Cano *et al.* (1992*b*), but they did not report the sequence for this overlapping region. Consequently, we were not able to make a direct comparison between our sequences and those of the previous study.

Phylogenetic analyses of these sequences together with four fungal, two vertebrate and six insect sequences, show that even for the shortest sequences of only 84 bp, the non-dipteran insects, including three hymenopterans, are resolved, with high bootstrap support, as a monophyletic group which excludes all of the cloned sequences (figure 3). In all three analyses the cloned sequences group with the vertebrate or fungal sequences. Several sequences from insect extracts are shared with those from resin extracts, and the phylogenetic analyses show that both sets of sequences are derived from similar, non-insect sources and are the result of a contamination of the specimen, DNA extraction or PCR reaction by vertebrate and fungal DNA.

4. DISCUSSION

We have applied a wide range of extraction and PCR amplification techniques to 15 fossil insects preserved in amber or copal but have failed to recover any authentic ancient insect DNA. Our negative results are in conflict with previous claims of ancient DNA extraction from amber-preserved insects. Before concluding that the recovery of ancient insect DNA from resin-entombed fossils is not reproducible we have to consider and discount other possible explanations for our negative results: inadequate sampling and inappropriate methodologies.

First, if only a minority of all amber-preserved organisms contain amplifiable ancient DNA, then our survey may not have examined a sufficient number of specimens. Previous studies of ancient DNA from archaeological and museum specimens have shown a great variation in the proportion of samples that yield authentic sequences (Pääbo 1989; Höss *et al.* 1996). All claims of ancient DNA from amber-preserved animals and plants report almost 100% success rate, with a total of 13 out of 14 specimens yielding apparently authentic sequences. We have examined more specimens than in all six previous studies combined, including ten specimens of a single species, *P. dominicana*.

Second, we may not have examined a sufficient range of insect specimens. This also seems unlikely as

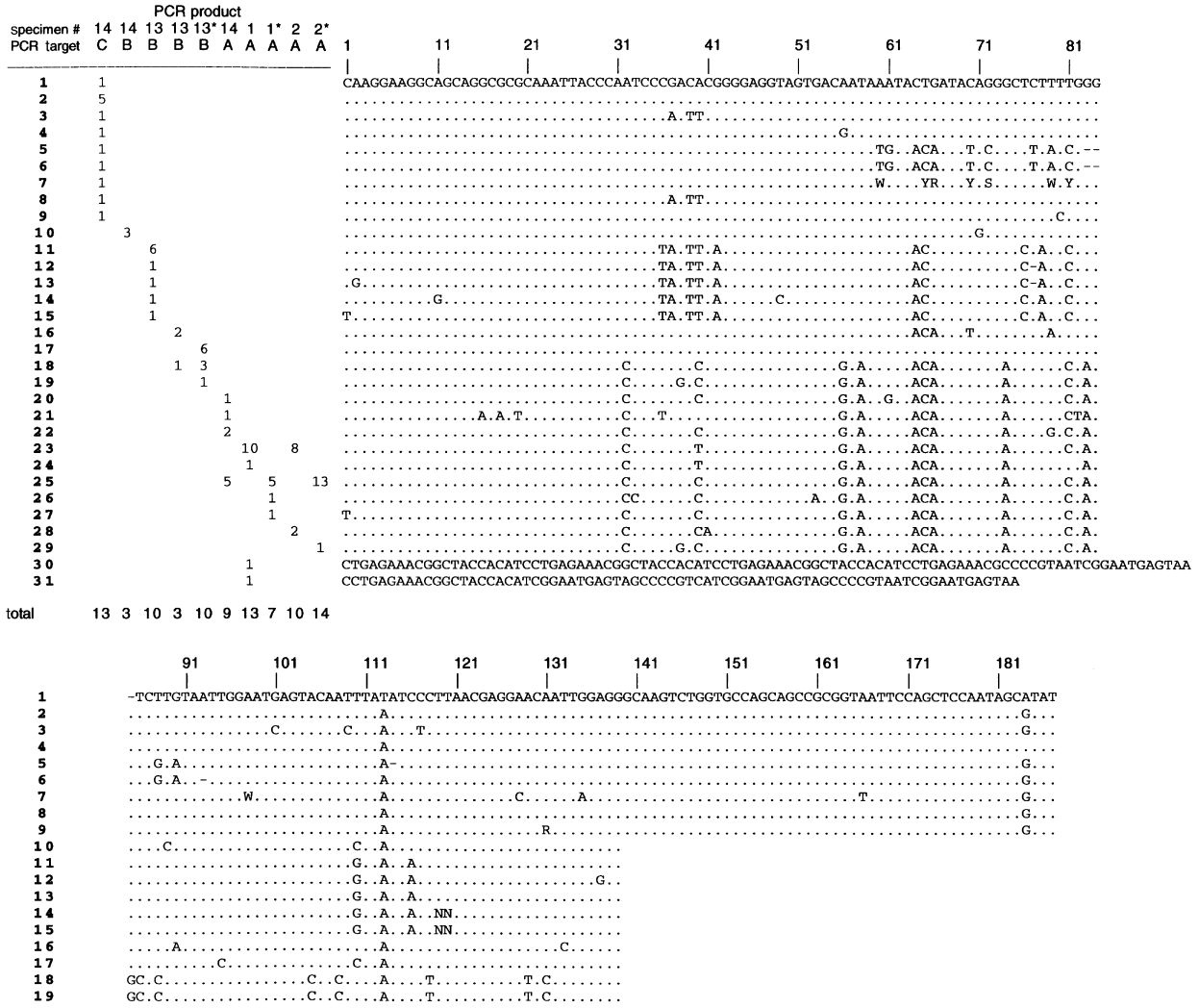

Figure 2. DNA sequences, and their frequency of occurrence, of cloned PCR product from amber- and copal-preserved bees. PCR products are identified by the specimen number and PCR target letter. Asterisks indicate PCR product amplified from extracts of fossil resin only (no insect tissue). Sequences 30 and 31 are composed entirely of tandem repeats of the forward or reverse primers b and f. Dots indicate identity with the first sequence.

we have studied three different insect taxa, including *P. dominicana*, the focus of the first claim of ancient DNA from amber-preserved organisms, of different body sizes, preserved in different fossil resins of different age and origin. These cover and exceed the range of taxa, ages and resins in all previously published claims.

Third, our DNA extraction and PCR assays may have been insufficiently sensitive to detect any authentic ancient DNA that may be present. We have tested five different DNA extraction methods, which have been used with apparent success on a range of ancient specimens. We have used a range of PCR conditions and primers, optimized to detect in the order of 10 template molecules. PCR targets were of the same or much smaller size than in all previous studies of amber-preserved organisms (figure 1), and were consistent with the small size of DNA fragments amplifiable from other ancient specimens (Pääbo *et al.* 1989; Cooper 1994; Handt *et al.* 1994). PCR attempts have targeted the same conserved regions of the nuclear small subunit and mitochondrial large subunit rRNA genes as those in previous studies of amber-preserved insects (figure 1).

The lack of reproducibility of ancient DNA sequences from amber-preserved insects leads us to question the authenticity of sequences reported in previous studies, on the basis of a number of inconsistencies. First, PCR fragments in excess of 500 bp were amplified from a Dominican amber bee and weevil (Cano *et al.* 1992*b*, 1993). The length of these PCR products is in contrast to the much shorter fragments, in the order of 200 bp or less, that are amplifiable from much younger archaeological specimens (Pääbo *et al.* 1989; Cooper 1994; Handt *et al.* 1994). In fact, the ability to amplify long PCR fragments is often used as an indication of contamination by contemporary DNA (DeSalle *et al.* 1992; Handt *et al.* 1994). In addition, these long sequences are not consistent with the sequences from two fossil termites, also from Dominican amber, that were amplified by 'jumping' PCR (Pääbo *et al.* 1989; DeSalle *et al.* 1993), a strong indication that there was no DNA template in the extracts longer than the 225 bp PCR target.

Second, despite amplifying relatively long sequences, previous studies have reported, on average, only 61% and as little as 28% (157 bp of a 556 bp fragment) of

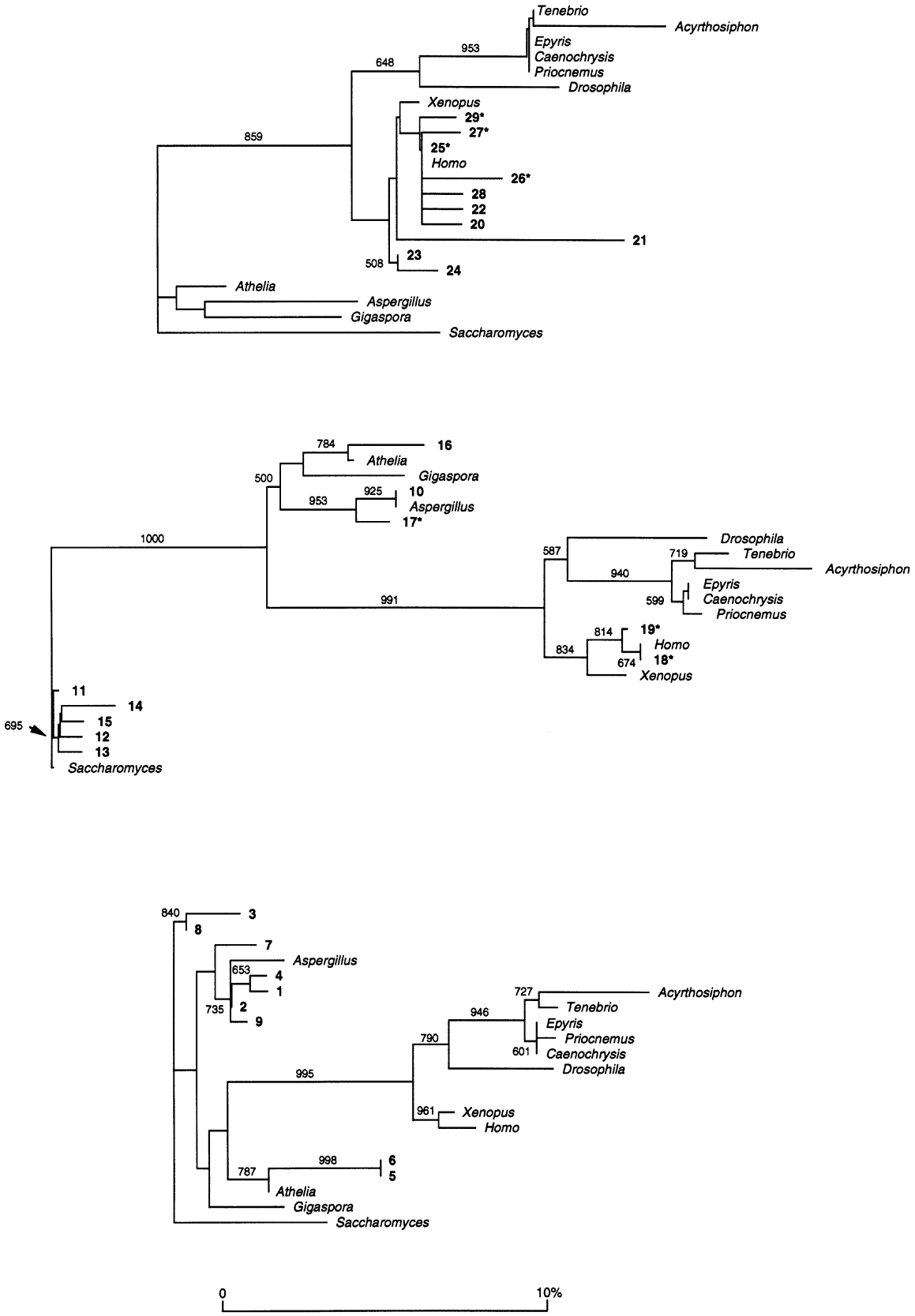

Figure 3. Phylogenetic relationships of DNA sequences from amber- and copal-preserved bees, inferred using the neighbour-joining algorithm. The trees were rooted using *Saccharomyces cerevisiae* as an outgroup. Numbers adjacent to branches refer to bootstrap values (greater than 500) from 1000 replications. The three trees are based on sequences from the 84 bp, 139 bp, and 187 bp PCR fragments A, B and C. Asterisks indicate sequences obtained from extracts of fossil resin (no insect tissue).

the amplified sequence. One possible explanation for the failure to report full length sequences is poor quality and/or ambiguous sequencing reactions, which must place doubt on the accuracy and validity of the sequences that were reported.

Whereas ancient DNA sequences from specimens younger than 100000 years old have now been replicated independently (Hagelberg *et al*. 1994; Höss *et al*. 1994; Taylor 1996), we have singularly failed to recover authentic ancient DNA from amber fossils. Additionally, attempts to obtain insect DNA from more than 30 insects preserved in Dominican (Howland & Hewitt 1994) and Baltic (Pawlowski *et al*. 1996) amber have been unsuccessful. The incompatibility between these and our negative results and previous reports of ancient DNA from amber-preserved insects is difficult to reconcile without suggesting some form of cryptic contamination in the latter. Contamination is a major problem in the retrieval of authentic ancient DNA sequences (Lindahl 1993; Handt *et al*. 1994).

The only sequences that we and others (Pawlowski *et al*. 1996) were able to detect were derived from obvious sources of non-insect contamination. More insidious forms of contamination from modern insects are very difficult to detect or rule out. Contaminating insect sequences have already been reported in studies of amber-preserved termites (DeSalle *et al*. 1993) and beetles (Howland & Hewitt 1994). DeSalle *et al*. (1993) identified at least three classes of contaminating sequences in DNA extracted from a fossil termite, two from extant insects, *Drosophila* and *Gryllus* (a genus of cricket) and a third, not positively identified, but which could have involved fungal or metazoan DNA or some chimera of both. Howland & Hewitt (1994) could find only contaminating sequences from a genus of grasshopper, *Chorthippus*, in extracts from two Dominican amber beetles. Despite extreme measures to prevent contamination by contemporary DNA, it obviously can and does occur (DeSalle *et al*. 1993; Handt *et al*. 1994; Howland & Hewitt 1994; Zischler *et al*. 1995; Pawlowski *et al*. 1996). In the absence of any independent replication of DNA sequences from amber-preserved insects we have to conclude that previous studies have been misled either by minute amounts of contaminating DNA or by the vagaries of molecular biological techniques acting on extremely small quantities of damaged DNA.

Although no negative result can disprove the existence of ancient DNA in amber-preserved fossils, our work shows that isolation of geologically ancient DNA from amber-preserved insects is not reproducible. Given the superb morphological and biochemical preservation of these fossils they were thought to represent the best circumstances for obtaining genetic sequence data from geologically ancient specimens. Neither amber nor much younger copal specimens have yielded positive results. Our negative results support the conclusion of others (Handt *et al*. 1994; Hedges & Schweitzer 1995; Soltis *et al*. 1995) that, in the absence of unambiguous and independent verification, research on geologically ancient DNA will remain little more than a 'biological curiosity'. In addition, the entirely destructive nature of current DNA extraction techniques and the paucity of significant biological questions addressed by molecular-based studies of amber-preserved organisms to date, lead us to suggest that the primary value of amber-preserved fossils lies in their excellent morphological preservation and not in the fragmented remains of any DNA whose existence remains speculative at best.

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