The invention of the polymerase chain reaction (1) has made it possible to determine DNA sequences from remnants of extinct species and past populations (2, 3). In addition, recent reports have claimed that DNA can be retrieved from paleontological finds that are millions of years old (4). However, because only a minority of ancient specimens contain amplifiable ancient DNA (5), false positives resulting from minute amounts of contaminating DNA pose a serious threat (6, 7). Although several ways to authenticate ancient DNA have been suggested (2, 6, 8), the field is in need of techniques that can indicate whether a particular ancient specimen may contain endogenous nucleic acids.

All amino acids used in proteins, with the exception of glycine (Gly), can exist in the form of two optical isomers, the D- and L-enantiomers, of which the L-enantiomer is used exclusively in protein biosynthesis. Once isolated from active metabolic processes, the L-amino acids undergo racemization to produce D-amino acids until eventually the L- and D-enantiomers of a particular amino acid are present in equal amounts. The rate at which racemization takes place differs for each amino acid and is dependent on the presence of water, the temperature, and the chelation of certain metal ions to proteins (9). Racemization is thus affected by some of the same factors that affect depurination of DNA, the major hydrolytic reaction responsible for the spontaneous degradation of nucleic acids (10). The racemization of aspartic acid (Asp), which has one of the fastest racemization rates, has an activation energy and rate constants over a wide temperature range (at neutral pH) that are similar to those for DNA depurination (10, 11). To test whether the extent of amino acid racemization is a useful indicator of the extent of DNA degradation in ancient specimens, we examined archaeological specimens from which DNA sequences have been retrieved (12). In order to ensure as far as possible that the samples used yield genuinely ancient DNA, we limited our analysis to nine cases that fulfill a number of criteria of authenticity (2, 6, 8) and we excluded human remains because of the inherent difficulty of recognizing contamination from contemporary humans (2, 6, 8). We also analyzed 17 samples, including some human samples, from which no ancient DNA sequences could be amplified.

No DNA sequences could be retrieved from samples in which the D/L Asp ratio was higher than 0.08 (Table 1), whereas all samples with D/L ratios below 0.08 yielded DNA sequences. Furthermore, there was a rough relation between the extent of Asp racemization and the length of the retrievable DNA sequences (Fig. 1). In samples in which the extent of Asp racemization was similar to that caused by the 6 N HCl hydrolysis procedure (D/L = 0.05), sequences between 140 and 340 base pairs (bp) could be amplified, whereas samples with greater amounts of racemization tended to yield only shorter DNA fragments.

No general correlation was observed between the age of the samples and the retrieval of DNA or the extent of racemization. However, of the nine samples that yielded DNA, seven stemmed from cold environments and four of them have been shown to contain smaller amounts of DNA damage than samples that do not yield amplifiable DNA sequences (13). On the basis of the racemization half-lives of Asp reported for bone in various climatic regimes (9), the finding that an Asp D/L ratio of about 0.1 is the limit for the retrieval of useful DNA sequences implies that the survival of DNA is limited to a few thousand years in warm regions such as Egypt and to roughly 100 years in cold regions. Such temporal limits for DNA retrieval are similar to those predicted from laboratory experiments (10).

Aspects of amino acid preservation other than racemization do not show any correlation with DNA preservation (14).

Because the racemization of Asp is faster than that of other amino acids (9, 11), the extent of racemization of Asp, among the amino acids analyzed here, should be the greatest, followed by alanine (Ala) and leucine (Leu), if all amino acids are of the same age. In contrast, a D/L ratio for Asp that is lower than that for Ala or Leu indicates contamination by more recent amino acids. For the samples from which ancient DNA sequences could be retrieved (Table 1), the extent of racemization of Asp was always greater than that for Ala and Leu, however, no authentic DNA sequences could be retrieved from samples in which the racemization of amino acids did not follow this pattern. Thus, amino acid racemization provides a way to identify the large majority of ancient samples that are not expected to yield any ancient DNA. The usefulness of this technique is enhanced by the fact that samples of only a few milligrams are sufficient for the analysis, and the results can be obtained in only a few days.

Ancient DNA sequences that are purportedly millions of years old have been reported from dinosaur bones, Miocene plant fossils, and amber inclusions (4). The D/L Asp ratio in the Utah dinosaur bone

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**Fig. 1.** Extent of Asp racemization plotted (as the logarithm of the D/L ratio of Asp) against the maximum length of DNA amplified (in base pairs).
from which DNA sequences were reported (4) is 0.21, and is thus higher than for remains from which endogenous DNA has been extracted (Table 2). Moreover, Ala is more racemized than Asp, an indication that the amino acids present in this specimen are a mixture of different ages. The poor preservation of the amino acids implies that no endogenous DNA should remain in this sample, which is in agreement with earlier results (7). The D/L Asp ratio of a Tyrannosaurus rex bone found in Montana (4) was 0.23, and Ala was more extensively racemized than Asp. Two dinosaur specimens from Antarctica, a cold depositional environment, also indicate substantial racemization as well as the presence of contaminating amino acids (Table 2). Thus, the prospects of retrieving DNA sequences from dinosaur fossils seem bleak.

Table 1. The extent of racemization of Asp, Ala, and Leu and DNA amplifi-
ability for 26 archaeological and paleontological samples. DNA was extracted, amplified, and sequenced as described in the references. Briefly, for nonhu-
man samples 140 bp of the mitochondrial 16S ribosomal DNA (rDNA), or 120 bp of the mitochondrial 12S rDNA, were amplified, whereas for human sam-

Table 2. Racemization of Asp, Ala, and Leu in paleontological samples from which DNA sequences have been reported. Samples for which the D/L ratios were difficult to determine because quantities of amino acids were too small are indicated as ND (20). The Utah and Montana dinosaur samples are identical to those from which DNA sequences have been reported (4) or in which the presence of DNA has been reported in the press. The Clarkia and amber specimens were similar to samples from which reported DNA sequences (4) stem. Ma, million years ago; ka, thousand years ago; ND, not determined. Gly/Asp ratios are given for bone samples only.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age (Ma)</th>
<th>D/L Asp</th>
<th>D/L Ala</th>
<th>D/L Leu</th>
<th>DNA (bp)</th>
<th>Gly/Asp</th>
</tr>
</thead>
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<td>Dinosaur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrannosaurus rex (Montana)</td>
<td>65</td>
<td>0.23</td>
<td>0.59</td>
<td>ND</td>
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<td>0.27</td>
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<td>Dinosaur bone (Utah)</td>
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<td>0.21</td>
<td>0.91</td>
<td>ND</td>
<td>140</td>
<td>0.01</td>
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<tr>
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<td>0.16</td>
<td>0.16</td>
<td>ND</td>
<td>67</td>
<td>0.33</td>
</tr>
<tr>
<td>Ornithomimidae (Antarctica)</td>
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<td>0.44</td>
<td>0.48</td>
<td>ND</td>
<td>1.28</td>
</tr>
<tr>
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<td>0.02</td>
<td>0.01</td>
<td>ND</td>
<td>0.00</td>
</tr>
<tr>
<td>Clarkia leaf 1</td>
<td>17</td>
<td>0.15</td>
<td>ND</td>
<td>0.00</td>
<td>140</td>
<td>0.00</td>
</tr>
<tr>
<td>Clarkia leaf 2</td>
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<td>0.29</td>
<td>ND</td>
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<td>0.00</td>
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<td>ND</td>
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<td>0.15</td>
<td>ND</td>
<td>0.00</td>
<td>200</td>
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</tr>
<tr>
<td>Clarkia sediment 2</td>
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<td>0.14</td>
<td>ND</td>
<td>0.00</td>
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<td>Amber</td>
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<td>0.01</td>
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<td>140</td>
<td>0.00</td>
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<tr>
<td>Diptera (Baltic Sea)</td>
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<td>0.05</td>
<td>0.01</td>
<td>0.00</td>
<td>140</td>
<td>0.00</td>
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<table>
<thead>
<tr>
<th>Sample</th>
<th>Age (10^7 years ago)</th>
<th>D/L Asp</th>
<th>D/L Ala</th>
<th>D/L Leu</th>
<th>DNA (bp)</th>
<th>Gly/Asp</th>
</tr>
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<td>Equus sp. (California)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
<td>0.00</td>
<td>340</td>
<td>0.27</td>
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<tr>
<td>Mylodon darwini (Chile)</td>
<td>13</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
<td>200</td>
<td>0.00</td>
</tr>
<tr>
<td>Mammutthus primigenius (Yuribe, Siberia)</td>
<td>9.7</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
<td>200</td>
<td>0.00</td>
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<td>Equus ferus (Siberia)</td>
<td>42</td>
<td>0.06</td>
<td>0.01</td>
<td>0.00</td>
<td>200</td>
<td>0.00</td>
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<tr>
<td>M. primigenius (Khastanga, Siberia)</td>
<td>50</td>
<td>0.06</td>
<td>0.01</td>
<td>0.00</td>
<td>200</td>
<td>0.00</td>
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<td>0.06</td>
<td>0.01</td>
<td>0.00</td>
<td>200</td>
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<td>13</td>
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<td>0.04</td>
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<td>0.00</td>
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<td>0.20</td>
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<tr>
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<td>0.02</td>
<td>0.00</td>
<td>0</td>
<td>0.10</td>
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<tr>
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<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>Human femur (Egypt)</td>
<td>4.5</td>
<td>0.29</td>
<td>0.01</td>
<td>0.00</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>Human femur (Egypt)</td>
<td>4.5</td>
<td>0.29</td>
<td>0.12</td>
<td>0.00</td>
<td>0</td>
<td>0.16</td>
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<tr>
<td>Human femur (Egypt)</td>
<td>4.5</td>
<td>0.30</td>
<td>0.00</td>
<td>0.00</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>Human femur (Egypt)</td>
<td>4.5</td>
<td>0.31</td>
<td>0.02</td>
<td>0.00</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>Mevalonix sp. (Florida tooth)</td>
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<td>0.33</td>
<td>0.44</td>
<td>0.23</td>
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<td>0.34</td>
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<td>0</td>
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<tr>
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<td>0.81</td>
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<td>0</td>
<td>0.61</td>
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<tr>
<td>Eremotherium mirabile (Peru)</td>
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<td>0.27</td>
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</tr>
<tr>
<td>Megalocnus sp. (La Brea, California)</td>
<td>15</td>
<td>0.75</td>
<td>0.53</td>
<td>0.24</td>
<td>0</td>
<td>1.89</td>
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</tbody>
</table>

![Image](https://via.placeholder.com/150)
lower than that of the insect tissue (17), the amino acids detected are likely to be endogenous. The surprising preservation of the amino acid stereochirality in amber-entombed insects may be due to the anhydrous nature of the amber matrix. Because depurination of DNA would similarly be inhibited by anhydrous conditions, an amber matrix may provide conditions conducive to the long-term preservation of nucleic acids.

REFERENCES AND NOTES

12. External surfaces of bone sections (~1 mm) were removed, and samples were ground under liquid nitrogen in a Freezer/Mill 7000 bone grinder (Spex Industries, Edison, NJ). Then 0.1 to 0.5 g were hydrolyzed in doubly distilled 6 N HCl for 24 hours at 100°C. Glassware was cleaned by immersion in 10 M HCl for 2 weeks, then rinsed in doubly distilled water and baked at 250°C for 1 week. Soft tissue samples were briefly rinsed in 0.1 N HCl and hydrolyzed as above. Subsequently, samples were dried under vacuum over NaOH. Bone samples were redissolved in doubly distilled water and desalted with a cation exchanger (50W-X8) (Bio-Rad) as in (17). B. L. Bada, Earth Planet. Sci. Lett. 15, 223 (1971). Soft tissue samples were dissolved directly in 0.1 N HCl, and amino acids were derivatized with O-phenyldimaleic acid/n-acetyl-L-cysteine (OPA/NAC) and analyzed by high-pressure liquid chromatography (HPLC) (Gilson, Middleton, WI) with previously described reagents that were used as the standards. To determine the extent of racemization caused by the experimental procedure, we analyzed five bovine serum albumin (Pharmacia) samples; they were found to have a D/L ratio for Asp of 0.034 ± 0.005.

Variation due to sample processing was tested by the analysis of 10 samples from the same bone, yielding a D/L ratio of 0.056 ± 0.0006. We investigated the relative fluorescence of OPA-derivatized D and L forms of Asp, Ala, and Leu by analyzing samples with D/L ratios of 1. The observed ratios (0.867, 0.85, and 0.83, respectively) were used to adjust values determined for the samples.


14. For example, the total amounts of the six amino acids analyzed [Asp, serine (Ser), Ala, Gly, Leu, and valine (Val)] do not correlate with the retrieval of DNA sequences (H. N. Poirar, M. Hös, J. L. Bada, S. Pállo, data not shown). Furthermore, a ratio of Gly to Asp of 5.5 or larger can be used as a rough estimate of the preservation of collagen in bone (H. Elster, E. Gil-Av, S. Weiner. J. Arch. Sci. 18, 505 (1991). However, DNA could be extracted both from bones in which collagen was preserved according to this criterion and from some in which it was not.

15. Only minute amounts of amino acids were present in these samples, and these values were not significantly different from those in the surrounding sediment. As a result, the D/L ratios for Asp could not be determined in most cases. The presence of bacterial amino acid decomposition products, for example, β-alanine and γ-amino-n-butyric acid, in some specimens also suggests bacterial contamination (J. L. Bada, unpublished work).


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Polymers with Very Low Polydispersities from Atom Transfer Radical Polymerization

Timothy E. Patten, Jianhui Xia, Teresa Abernathy, Krzysztof Matyjaszewski*

A radical polymerization process that yields well-defined polymers normally obtained only through anionic polymerizations is reported. Atom transfer radical polymerizations of styrene were conducted with several solubilizing ligands for the copper(I) halides: 4,4'-di-tert-butyl, 4,4'-di-n-heptyl, and 4,4'-di-[5-(nonyl)-2,2'-dipyridyl]. The resulting polymers have all of the characteristics of a living polymerization and displayed linear semilogarithmic kinetic plots, a linear correlation between the number-average molecular weight and the monomer conversion, and low polydispersities (ratio of the weight-average to number-average molecular weights of 1.04 to 1.05). Similar results were obtained for the polymerization of acrylates.

Szwarc and Levy first reported on living anionic polymerizations in 1956 (1, 2), and this technique made it possible to prepare well-defined polymers. A living polymerization is a chain-growth polymerization that propagates with no irreversible chain-transfer or chain-termination reactions. Provided that initiation is fast, the degree of polymerization is proportional to the ratio of the concentrations of monomer consumed and initiator, and the product polymer has a polydispersity that approaches a Poisson distribution. The discovery of living polymerizations revolutionized synthetic polymer chemistry, because with such polymerizations one can prepare block and triblock copolymers, end-functionalized polymers, and star polymers (3). Of the known living ionic and metal-catalyzed polymerizations, living anionic polymerization affords the best control over the resulting polymers and provides polymers with the narrowest molecular weight distribution (ratio of the weight-average to number-average molecular weight, M_w/M_n).

Radical polymerizations are important for the industrial production of commodity polymers, which account for a major fraction of polymer industry's annual production, yet are very difficult to control because of fast, irreversible termination of the growing radicals through coupling and disproportionation reactions. Because of these