Amplification of mitochondrial DNA from preserved scorpions.

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Scorpions are the most ancient group of terrestrial animals and confound phylogenetic analyses through a lack of "good" morphological characters.

Many museum specimens are a valuable source of genetic information, although a critical factor is the degree of DNA degradation within the specimen. Scorpions are never pinned and dried, and most preserved material is stored in 4% formalin or 70% alcohol. The penetration of fixative into the scorpion body is usually slow, evidently due to the epicuticular lipid deposits which prevent water loss in scorpions, especially in desert species. In the 1970s, scorpion taxonomists started to use heat shock treatment (dipping in boiling water) to kill specimens, fixation in AFA (85 % ethanol, 10 % formalin, 5 % glacial acetic acid) for 12-24 hours, with subsequent storage in alcohol (Sissom *et al.* 1990). Our goal was to study the degree of DNA degradation due to storage in different fixatives and to amplify genes of mtDNA from this material. We were particularly concerned about the effects of AFA as a fixative as it contains acetic acid. We used old collections of *Euscorpius mingrelicus* (Caucasus; stored in 70% ethanol for seven years) and *Centruroides exilicauda* (Baja California; stored in 70% ethanol for seven years) as well as new collections of *Hadrurus concolorous* (Baja California), which were stored for 14 days in the following solutions; 70% and 96% ethanol, 70% and 100% isopropanol, 5% formalin and AFA. Scorpions were dismembered so that fixative would penetrate tissues completely. Other specimens were Silicagel-dried and dried at ambient temperature. DNA extracted from live scorpions was used as a control.

DNA was extracted from metasomal segments ("tail") or pedipalps ("pincers") which were minced, rinsed in water, and ground in liquid nitrogen. We followed a standard total DNA extraction protocol with DTT buffer (Thomas *et al.* 1990) and a PCR protocol (Simon 1991) with 12S rRNA *Drosophila* primers (Poindexter and Fet 1992) and cytochrome oxidase I (degenerate) primers (P. Sudman, pers. comm.) for mtDNA. The authenticity of amplified DNA (300 to 400 bp fragments) was confirmed (Sudman, Fet, and Vezzetti, in prep.) by comparing sequences obtained through asymmetric amplification with known scorpion 12S sequences (*Liocheles*; Ballard *et al.* 1992) and *Drosophila* cytochrome oxidase I sequences (Clary and Wolstenholme 1985).

The highest DNA yield and the least degraded DNA were obtained from 70% and 96% ethanol specimens while DNA from isopropanol gave a good yield but was significantly more degraded. Yields of DNA from formalin and AFA was very low; ambient-dried specimens yielded much more degraded DNA than Silicagel-dried ones. However, all extractions (except ambient-dried and isopropanol-fixed specimens) produced strong, clean PCR products even when the yield from DNA extraction was very low (including material stored in 5% formalin and AFA, as well as seven year-old AFA material). For the preservation of high molecular weight DNA, high yields and successful mtDNA PCR amplification we recommend storage of scorpions in 96% ethanol. This conclusion is consistent with the opinion of Post (1992) that less degraded DNA is obtained from insects stored in ethanol than isopropanol.

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