Chapter 31
Stable isotope analysis of seeds and bones

Appendix 31.01. Stable isotope analysis laboratory protocols

Seed preparation

All of the emmer caches were of spikelets (grain still encased in chaff) except for cache <1164> which comprised cleaned emmer grain with hulled barley florets. The flax cache also comprised cleaned seeds. In all cases, spikelets, grains or seeds were collected at widely spaced intervals across a cache surface, to reduce the chance of sampling from the same original cereal ears or flax capsules. Each selected item was digitally photographed using a Leica stereozoom M205 microscope, including both prior to and after the removal of chaff from the emmer grains recovered within spikelets.

Seeds were chemically pretreated for isotopic analysis with an acid wash to remove secondary carbonates. The grains were soaked in 0.5M aq. hydrochloric acid (HCl) solution at 70°C for 1 hour to remove carbonates. Each grain was rinsed three times in distilled water and then freeze dried. The grains were then crushed individually and weighed for analysis.

Bone collagen extraction

Collagen was extracted from the bone samples following a method described in Richards & Hedges (1999). *c*. 0.5g of bone was sampled using a hand-held drill with a diamond cutting wheel and then sandblasted to remove surface dirt. The samples were demineralized in 0.5M aq. HCl for up to 2 weeks at 4°C, rinsed and then gelatinized at 75°C for 48 hours in pH 3 water. The 'collagen' was then filtered and then lyophilized before weighing for isotopic analysis. Each collagen sample was measured in triplicate.

Isotopic analysis

Samples were isotopically analysed using a Costech elemental analyser coupled in continuous flow mode to a Finnigan Delta V isotope ratio mass spectrometer. Carbon and nitrogen isotopic ratios are expressed as a delta value ($\delta^{13}C$ and $\delta^{15}N$) relative to international scales (VPDB and AIR, respectively; Craig 1957; Mariotti 1983). Repeated measurements on international and in-house standards showed that the analytical error was less than 0.2‰ for both carbon and nitrogen.

Measured collagen is deemed to be of good quality if it fulfils the following criteria: an atomic C:N ratio of 2.9–3.** (DeNiro 1985); a 'collagen' yield of 1% by mass; final carbon yields of 13%; and final nitrogen yields of 4.8% (Ambrose 1990). One animal sample (MF58, pike) failed to meet these criteria. The remaining samples all produced data deemed to be of good quality. Collagen yields were in excess of 3%, carbon yields > 20%, nitrogen yields > 7%. C:N ratios between 3.1 and 3.5.

References

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