# Use of Biological Collections

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The basic methodology for identifying biological remains from archaeological sites ("biodata") relies on comparison of unknown material with specimens of known identity. Such known specimens exist in biological collections, held, for example, in museums and archaeological science laboratories. The term "reference collection" is often used for the latter, with the implication that the material is specifically for comparison. Common types of reference collection include seeds, pollen, phytoliths, wood and vegetative anatomy, animal bones, teeth and antlers, animal hair and skin, shells, and insects. Although still primarily gathered for morphological and anatomical comparison, biological collections have also become important as a source of DNA and for other chemical analyses, such as stable isotope investigations.

The initial sorting and identification of biodata can be carried out using printed and online manuals, but actual reference materials are essential to verify and determine more difficult identifications. Reference collections are also used to identify subsidiary parts of an organism, for example, cereal chaff or fruit stalks; and specimens with detailed provenance can give further insights, for example, teeth from knownaged animals can be used to age archaeological specimens.

National and local museums can contain many millions of biological specimens. These collections may not be designed as reference collections for use within archaeology, but they remain an essential resource for specialist work. Full acknowledgment and citation of any specimens used as part of archaeological research should be given. Curators can also provide advice on collections care.

## Forming a reference collection

Collecting and curating reference material can be time-consuming and expensive. A long-established laboratory may have thousands of specimens, many gathered directly from the field, with dedicated staff time for curation. A new laboratory or independent researcher will often start by focusing on common taxa. The concept of a "distributed collection" is increasingly being explored (e.g., Fairnell and Orton 2017) in which collections are owned and housed individually, but are databased centrally, enabling workers in one institution to locate relevant material in others.

The range of taxa to be included in a reference collection will depend on the geographical study area and time frame of interest. The biota found in the region of interest today is the obvious starting point, but over time, different species will have been present. It may therefore be necessary to look further afield to find relevant comparable, surviving biotas.

The focus of a collection will also depend on the expected preservation of archaeological material; for example, if waterlogged or desiccated plant remains are likely, a wide range of vegetative plant parts will be required, in addition to fruits and seeds. If agricultural sites are the focus, then good representation of crop varieties and farm animal breeds will be desirable. Collection of reference material can be easily integrated with ethnoarchaeological work, for example, collecting by-products of crops or animal-carcass processing.

## Obtaining specimens

Reference material should be accurately identified and in good physical condition. Plant parts

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such as seeds and wood are ideally vouchered by a herbarium (pressed plant) specimen bearing leaves, flowers, and so on, that can be identified by a botanist and be available for future taxonomic investigations. An insect can be preserved whole and form its own voucher. For animals such as mammals and birds, a digital image of the carcass is required before preparation.

Fieldwork for acquisition of reference material should be planned to optimize the timing, for example, to coincide with flowering season or breeding condition. Protected areas, such as nature reserves and official enclosures, can be very productive. Collecting in the vicinity of an excavation has the benefit of increased understanding of the geomorphology and vegetation, which will be valuable in interpreting biodata.

Reference material can also be acquired or donated from existing collections, for example, botanical gardens or gene banks, although the quality of such specimens should be assessed. With such material, it is advisable to have two or three different accessions for a species so they can be checked for consistency in identification. Good documentation is important; confirmation should be sought that the material offered was legally acquired and is the property of the donor.

# Ethical and legal aspects

Collecting or exporting plants and animals may require permission from national or local representatives. Plants and animals listed by the Convention on International Trade in Endangered Species (CITES) will usually require both an export and import permit. When collecting is planned, it is advisable to get advice from local museums and field biologists. Collectors should also be aware of ethical aspects of collecting, for example, relating to rare plants and animals or recording of indigenous knowledge.

# Preparation and storage

## Plants and fungi

Plants are usually vouchered by a herbarium specimen. Macroremains such as seeds, fruits, and vegetative parts are collected into paper or cloth bags so that they can dry without going moldy. Fruit stones embedded in fleshy fruits should be extracted before the fruit dries. Once plant materials are dry, they should be frozen at minimum -20 °C for one week to kill any insect infestation. Seeds are usually kept in glass tubes or archival (e.g., polystyrene) plastic boxes in shallow drawers to allow easy browsing. Woods may be sectioned for anatomical slides, or charred for examination under an epi-illuminating microscope. Waterlogged archaeological material can be stored for reference use, either freeze-dried (risking some loss in definition) or wet in 70 percent ethanol or industrial methylated spirits (IMS), or sectioned on microscope slides using suitable mountants.

Microremains are usually also held on microscope slides, using permanent or semipermanent mountants. Starch granules (see STARCH GRANULE ANALYSIS) can be extracted from dry plant material by grinding. Pollen can be extracted from fresh or dry flowers using a brush. Phytoliths (see PHYTOLITH ANALYSIS) are extracted by ashing plant material in a muffle furnace, or treating with acid in a suitably equipped laboratory.

Plant DNA is usually extracted from leaves or seeds. It is routinely extracted from herbarium specimens up to 200 years old. However, if DNA extraction is desired from new collections, fresh leaves should be collected into paper bags, then packed in sealed plastic bags with silica gel to maintain a dry environment. If plant collections are treated with alcohol this will destroy any DNA within (Doyle and Dickson 1987).

#### Vertebrates

Animals should be identified to species, sex, and age, before the carcass is prepared for its skeletal and/or other relevant parts. Fresh carcasses of known animals are best, because they are easier to identify and handle.

The four main methods of bone preparation are burial, cold-water maceration, hot-water maceration, and dermestid beetles. The choice of which method to use often involves a compromise between time and smell. The quicker processes, hot-water maceration and beetles, can be the smelliest. Enzymes, such as Neutrase® and trypsin, can be used to help break down

the protein (flesh); biological washing detergents (which contain enzymes and mild bleaches) can be used to help break down protein and fat; fat can also be removed with the use of solvents, for example, acetone. Whichever method and chemicals are used, a risk analysis must be carried out beforehand to make sure the preparation is carried out safely. Fresh carcasses may be a source of zoonotic disease, such as avian flu; dermestid beetles need to be housed and cared for appropriately; and all chemicals must be used safely, following, for example, the United Kingdom's Control of Substances Hazardous to Health (COSHH) Regulations 2002 guidelines. A well-ventilated area is likely to be a prerequisite.

If any flesh or fat remains on or in the bones, the collection will be susceptible to insect and mold damage and will degrade. Fluctuations in temperature and humidity can also damage bone. Ideally, clean, dry bones should be stored in a controlled environment, with some ventilation. If stored in plastic bags, the bags should be perforated. Depending on the preparation method used, the bones may become unsuitable for future DNA analysis.

#### Invertebrates

Accurate identification of, for example, mollusc and insect species from the fresh specimen using relevant identification keys is vital before they are processed. A risk analysis of all processes and chemicals used should be carried out. Insect and mollusc collections can comprise many small specimens, which need to be stored in suitable boxes and trays so that they are easily accessible but protected from rough handling and light. The storage environment should be controlled to provide some ventilation and avoid fluctuations in temperature and humidity.

Insects can be pinned or mounted on microscope slides, depending on their size. Insects, and many other biological reference materials, can also be preserved in fluid, but such specimens are not as easy to use as comparanda because they cannot be readily handled. Some chemicals used to relax insects for pinning or mounting them on slides may destroy DNA. Insect collections are prone to insect attack, while mollusc collections

can be affected by acidic fumes released from organic packing material. Molluscs can be stored dry in sealed plastic bags.

# Organization and cataloging

There are three ways to organize a reference collection: in an evolutionary sequence, placing related taxa close to each other; in an alphabetical system, requiring no specialist knowledge to locate taxa; or in an index collection, arranged by element and size. The choice of sequence is not always simple and advice should be sought from other laboratories or taxonomists. It is important that the correct biological nomenclature is used; this may be based on a national checklist or on an online resource such as the GBIF Backbone Taxonomy (GBIF Secretariat 2016).

A catalog of the collection, usually in the form of a database, should indicate where to find a specimen and comprehensive details of the specimen's provenance, including, for example, source location/habitat, age, life history, and cause of death. The set of fields will usually be similar to that in the Access to Biological Collections Data (ABCD) schema (TDWG Task Group 2007). The data should be atomized, for example, scientific names broken down into genus, species, and author fields, locality and country recorded separately. It is better to have a smaller number of fields that are consistently filled, than many fields that are mostly empty. Once provenance data have been added to a database, the original documentation (including permits) should be stored

When specimens are integrated into a collection, they need to be given a unique accession number. In skeletal collections, that number can be written on each element; for pinned insects, the number is written on a card label; the number can also be written on the bag or container housing the specimen. If previously held elsewhere, the original collector and collection accession number should be retained, and it is good practice to cite both in reports.

SEE ALSO: Archaeoentomology; Charcoal and Wood Analysis; Environmental Archaeology; Paleoethnobotany; Zooarchaeology

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