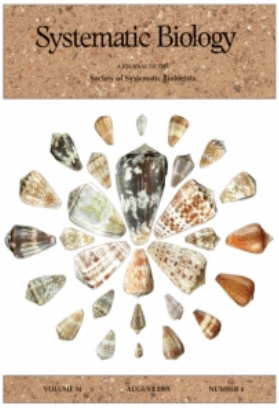


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# An Application of Tissue and DNA Banking for Genomics and Conservation: The Ambrose Monell Cryo-Collection (AMCC)

Angelique Corthals [a]; Rob Desalle [a]
[a] American Museum of Natural History, New York, New York, USA

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# Points of View

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## An Application of Tissue and DNA Banking for Genomics and Conservation: The Ambrose Monell Cryo-Collection (AMCC)

ANGELIQUE CORTHALS AND ROB DESALLE

*American Museum of Natural History, New York, New York 10024, USA; E-mail: corthals@amnh.org (A.C.)*

### THE NEED FOR TISSUE AND DNA BANKING IN SYSTEMATIC BIOLOGY

With the advent of the so-called genomic revolution and improved techniques of DNA analysis, combined with a rapidly vanishing biodiversity, the systematic community has been facing a remarkable—and often neglected—challenge for the past 50 years: to preserve genetic resources issued from research. The preservation and long-term storage of biological specimens' derived materials (e.g., DNA extracts) and associated data are essential to ensure comparability and reproducibility in all areas of biological research. Natural history museums and herbaria around the world are now in a position to face the exciting and challenging task of preserving the genetic library of life for generations to come. However, the lack and/or poor condition of preservation of molecular vouchers generated from often fragile and rare specimens have been problems too often underestimated or unable to be addressed due to lack of funding or, more pointedly, lack of interest in preservation of these important research materials.

The present article does not seek to reiterate the plea for genetic resource collections introduce by Dessauer et al. in 1984 (and more recently by Sheldon, 2001, and Savolainen and Reeves, 2004). It seeks to bring these collections, and the issues of preservation of genetic resources, to the awareness of the systematic biology community through the case study of the American Museum of Natural History new cryogenic repository, the Ambrose Monell Collection for Molecular and Microbial Research (AMCC; website: http://research.amnh.org/amcc/).

### OVERVIEW OF FROZEN TISSUE COLLECTIONS IN THE NATURAL HISTORY WORLD

There are five main sources that address the storage and curation of frozen tissue and/or genetic material existing in the United States and abroad (Prendini et al., 2002):

1. Culture and stock centers, where you can obtain live targeted species or strains, such as the *Drosophila* Species Stock Center (Tucson, AZ).
2. Cell line centers, where you can obtain cell lines for a wide variety of taxa, such as ATCC (American Type Culture Collection) and the ECACC (European Collection of Cell Cultures), or the CRES (Center for Reproduction of Endangered Species) at the San Diego Zoo.
3. Botanical and zoological gardens have always been an obvious source of genetic material for many varied taxa, and have made it possible to obtain DNA and tissue samples from traditional collections (such as Kew Gardens, or the South African National Botanical Institute, recently changed into the South Africa National Biodiversity Institute).
4. Seed and spore banks are important resources for genetic materials, in particular for cultivated species and their wild relatives, such as the U.S. National Plant Germplasm System (NPGS).
5. Frozen tissue collection from museums and other academic institutions remains the most important source of genetic material, because of their capacity of linking traditional vouchers (i.e., skeleton, skins, etc.) to tissue samples and their genetic derivatives (see Appendix 1 for a list of the main frozen tissue collections online).

Though latecomers in the field, museums are in a particularly significant position to lead the way in best practices of genetic resources management, because of their experience in curating traditional collections as well as in being able to link genetic samples to their traditional morphological vouchers.

Since the early pioneers of collecting fresh frozen tissues in the wild (Dessauer et al., 1986, 1996), many institutions have established frozen tissue collections (Sheldon, 2001). However, the drive to build such collections in natural history museums as a best method of preservation of genetic resources in the 1980s created

an ensuing number of issues that have been addressed only on sporadic—though notable—occasions, one of them being the special workshop panel of tissue collection managers, convened in 1983 at the Academy of Natural Sciences of Philadelphia (Dessauer and Hafner, 1984). But despite workshops, manuscripts, and actual set-up of model frozen tissue collections such as at Berkeley's Museum of Vertebrate Zoology, Louisiana Museum of Natural History, and the University of Alaska Museum of the North (see Dessauer et al., 1996; Sheldon, 2001; Prendini et al., 2002, for a list and history of existing collections), researchers in most institutions have typically kept on collecting frozen tissues as part of individual research projects, with resulting biomaterials stored within individual departmental laboratories suffering from eclectic history of acquisition, dispersed storage and inconsistent curatorial systems.

The "plea for DNA banking" recently published in Science by Savolainen and Reeves (2004) has shown that the issue of genetic resources collections, in particular frozen tissue collections, is still a subject of debate and has been somewhat neglected by those who need it the most: systematists and biological researchers of all ranks.

Among the issues stemming from the debate on genetic resources collections, I will address those which the newest kind of frozen tissue collections, such as the Ambrose Monell Cryo Collection, have tried to answer, namely, cryogenic versus ultracold, centralized versus noncentralized, tools and outreach, and the cost of building and running a cryogenic repository. Other issues exist, such as collection policies, compliance with the Convention on Biological Diversity, etc., but to approach these adequately would take a full article by itself. For these issues, and many more, I would refer the reader to Prendini et al. (2002) and the forthcoming publication by Kew Gardens on DNA banking (Savolainen et al., 2005).

## A Brand New Collection: Trying to Answer the Issues of Frozen Tissues

The Ambrose Monell Collection for Molecular and Microbial Research is the American Museum of Natural History's newest centralized cryogenics repository of tissues and DNAs originating from genomic research and legacy collections. Started in 2001 and funded by NASA, the collection currently houses over 30,000 tissue and DNA specimens frozen below −180°C in an array of vapor-phase liquid nitrogen vats. The AMCC is a visible example of a new kind of repository facility in the museum world, and is the first fully centralized cryogenic repository in natural history museums. Its model is based on biomedical and cell lines facilities (Eiseman and Haga, 1999).

### *Cryogenic versus Ultracold Freezers: How Cold Is Cold?*

When thinking of long-term preservation, colder is generally the better (Sheldon, 2001; Prendini et al., 2002). The great majority of collections in the museum settings, however, still use ultracold freezers rather than liquid nitrogen freezers (Hanner et al., 2005).

There are several essential reasons for the choice of preserving tissue specimens in an array of −180°C liquid nitrogen vapor phase such as in the AMCC (see Fig. 1). Mainly, the molecular integrity and quality of the samples is best served by this method of storage. Studies have shown that tissue specimens preserved at −20°C and −80°C are still subject to degradation (Florian, 1990). Furthermore, with specimens flash-frozen in the field using liquid nitrogen dryshippers, and maintained at temperatures below the glassification point of water (Franks, 1985), it is possible to virtually stop biological time and preserve a whole range of fragile molecules, such as RNA, which is particularly important given the unpredictability of the long term use of the samples. It is also possible to long-term preserve viable cells, which is otherwise impossible in temperature above −120°C.

Another important reason is that liquid nitrogen is by its own physical property cold, and therefore does not need mechanics or electricity to remain cold. Thus, a collection with liquid nitrogen freezers does not have to fear extended periods of power outage or mechanical failures.

### *Centralized versus Noncentralized*

Noncentralized collections are prone to a variety of problems, such as:

1. A variety of containers and labeling techniques have been used for making tissue collections. Many, but not all, containers are inadequate for long-term archival (vials with external threading, in particular, are susceptible to cracks and loss of air-tightness, eventually leading to desiccation and oxidation of the specimens and/or leakage). Moreover, many of the techniques for labeling are inadequate: numbers written on pieces of tape that were applied to collection vessels do not adhere properly under long-term storage conditions at low temperatures; in other cases the writing itself is disintegrating with changes in temperature associated with specimen sorting and retrieval, and/or buffer leakage.
2. Many tissue collections have been located in mechanical freezers without sufficient backup freezer space to handle meltdown of a malfunctioning freezer, or backup power in the case of extended service interruptions. Furthermore, by having an unrestricted and unguarded access to the freezers, tissue collections run a very high risk of disappearance, accidental thawing (sample left out by mistake), and contamination. In most cases, specimens used for research or sent out on loans are subsampled within laboratories, which house numerous polymerase chain reaction (PCR) machines and no biosafety cabinets. This increases severely the risk of contamination of the samples (through aerosol DNA), as shown by Scherczinger et al. (1999).

Many collections have tried to answer these issues in different ways, namely by partially centralizing their

FIGURE 1. The vat room (or cryo-storage room) at the AMCC.

collections and applying standards of labeling and packaging, as well as having an acting gatekeeper, though most have kept an ultracold mechanical freezers system, relying on battery and generator backups in case of power shortage (Sheldon, 2001; Prendini et al., 2002; Hanner et al., 2005).

The AMCC has tried to answer all these issues at once: to maintain samples in stable, liquid nitrogen–charged cryogenic freezers, addressing thus the problem of mechanical freezer meltdown and power outage, and to have a fully centralized collections in order to secure the samples physically and develop standards in sample preservation and transfer. Unlike most frozen tissue collections, the AMCC has been beneficiating from a high-level administrative decree from the American Museum of Natural History mandating that all departments deposit all their biological samples and byproducts into the new facility. The collections from different department held centrally in the AMCC are maintained and managed by a dedicated staff, but unlike many other collections do not have separate freezers and are not considered separate entities.

Furthermore, the cryorepository laboratory facilities are bio-safety level II and include a card-access lobby separated from the main laboratory area by an AMCC staff only card access–locked door. As a restricted centralized repository, the AMCC has also answered the problems of contamination avoided by the use of two biosafety cabinets in which all transfers are performed, and by the absence of PCR machines in the facilities.

### *The Instruments of the Passion: Tools of Collection Management*

For many small frozen tissue collections, the retrieval of samples relies critically on the memory of the collection manager and a single Excel spreadsheet or in the best case, access database, to locate specimens. Often, the electronic databases are not accessible to outside researchers. However, there lies one of the most critical issues of frozen tissue repositories that have seldom been addressed in the literature: the need for a reliable comprehensive database, not only as a day-to-day collection management tool, but also as a means to broadcast the collection's holdings to the scientific community at large.

Thus, in its daily operations, the AMCC tissue samples are indexed using Freezerworks Unlimited, a relational database application program well suited to the task of freezer inventory management (Ioannou, 2000). The program creates a record for each specimen giving it a unique barcode ID. Each record contains data ranging from the collecting event to each the position of each vial in the collection's many freezers. Following data entry, the program generates a printed cryoresistant label,

which includes the unique ID number both as a barcode as well as a human-readable numeric string. This feature allows for laboratory technicians to retrieve any vial from the freezers quickly and reliably by scanning the label to retrieve its associated data and thus confirm the identity of the specimen they are attempting to retrieve.

The collection holdings are made accessible to the scientific community worldwide through the facilities website (http://research.amnh.org/amcc/).

Furthermore, the database is designed to integrate with the National Center for Biotechnology Information's Entrez indexing and retrieval engine and its LinkOut service (http://www.ncbi.nlm.nih.gov/entrez/linkout/). This allows AMCC records with nucleotide sequence accession numbers to link out to corresponding pages on the NCBI Genbank and Taxonomy databases and inversely for GenBank sequences to link out back to the AMCC.

### *Cost of a Cryogenic Collection*

The AMCC is funded by a grant from NASA, which covers its daily operations as well as the salaries of four full-time staff (one curatorial associate, one collection manager, two technicians). The facilities cover 2000 square foot of the basement at the AMNH, and its repository has seven cryogenic freezers, a 3000-gallon bultank, and two different preparation labs.

There is no denying that the size of the collection and its annual running cost (without salaries, around US$80,000) are prohibitive for most small institutions in the United States as well as small institutions worldwide, in particular those situated in developing countries. This is why the AMCC has a program of partnerships with other institutions and offers also its services, from accessioning to loan shipping, even to outfitting research with field collection kits for free, so that the entire scientific community can benefit from the facilities.

## CONCLUSIONS AND RECOMMENDATIONS

Though often difficult to implement in large institutions, we strongly recommend the need for a centralized repository, combined with bioinformatics initiatives, as a way to solve problems of security, safety, and quality control.

Institutions around the world must assess the preservation state of their current specimens collections and their role in modern genetic and genomic studies. Museums, universities, herbaria, and zoos should come together to build a common platform, to ensure communication between repositories and those who need them. Such efforts are underway in many countries, and in institutions within the United States. It should be every repository's responsibility to ensure that they participate in nationwide programs for the preservation and use of genetic resources.

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APPENDIX 1. Frozen tissue collection links. This list is by no mean exhaustive, but offers a starting point for searches on frozen tissue collections (Herbaria not included) in academic institutions accessible through the Web.

| | |
|---|---|
| Ambrose Monell Cryo Collection | http://research.amnh.org/amcc/ |
| Humbold State University | http://www.humboldt.edu/~bsa2/collection.html#tissues |
| Louisiana State University | http://www.museum.lsu.edu/LSUMNS/Museum/NatSci/tissues.html |
| Museum of Southwestern Biology | http://nix.msb.unm.edu/test/queryform.php |
| Museum of the North, Alaska | http://www.uaf.edu/museum/af/ |
| Museum of Vertebrate Zoology, Berkeley | http://www.mip.berkeley.edu/mvz/collections/TissueCollection.html |
| Smithsonian National Museum of Natural History | http://www.mnh.si.edu/rc/ |
| South Australian Museum | http://www.samuseum.sa.gov.au/orig/ebu.htm |
| Texas A&M | http://wfscnet.tamu.edu/tcwc/tissue_collection.htm |
| The Field Museum, Chicago | http://www.fieldmuseum.org/research_collections/default.htm |
| The Natural History Museum, London | http://www.nhm.ac.uk/zoology/zoocollect.html |
| University of Washington, Burke | http://www.washington.edu/burkemuseum/tissuepolicy.html |

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# Measuring Support and Finding Unsupported Relationships in Supertrees

MARK WILKINSON,[1] DAVIDE PISANI,[1,2] JAMES A. COTTON,[1] AND IAN CORFE[1,3]

[1]*Department of Zoology, The Natural History Museum, London SW7 5BD, UK; E-mail: marw@nhm.ac.uk (M.W.)*
[2]*Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland*
[3]*Department of Earth Sciences, University of Bristol, Bristol BS8 1RJ, UK*

Supertree methods can combine information in phylogenetic trees to yield novel relationships, but matrix representation with parsimony (MRP) supertree methods (Baum, 1992; Regan, 1992) sometimes return supertrees that include relationships that appear to have no support among the input trees, individually or jointly (Bininda-Emonds and Bryant, 1998; Pisani and Wilkinson, 2002; Wilkinson et al., 2004). Assessing the extent to which this might occur in practice requires a clear conception of how a set of input trees may provide support for relationships in supertrees. Bininda-Emonds (2003) broke new ground in presenting the first explicit conceptual analysis and categorization of the kinds of correspondence that can occur between relationships in input trees and supertrees, and he investigated the frequency of unsupported relationships in some real supertrees and with simulations. He reported that unsupported clades were completely absent from the real supertrees and very rare in simulations, suggesting that unsupported groups are unlikely to be a problem for MRP in practice.

Here we present an alternative view of the correspondences between relationships in supertrees and input trees, and define associated measures that quantify these correspondences. We review previous work, contrast it with our own, and consider the implications. We draw heavily upon the treatment of analogous problems in the correspondence between characters and phylogenetic trees (Wilkinson, 1998). Following Bininda-Emonds (2003), we focus almost exclusively upon support for supertree clades (components, rooted full splits), as opposed to support for other relationships (e.g., resolved triplets, partial splits, nestings, subtrees) or nestings, but our approach readily generalizes to unrooted trees. We thus aim to clarify how a rooted input tree can support or conflict with a supertree clade. All reference to Bininda-Emonds is to his 2003 article, unless otherwise indicated.

## SUPPORT, CONFLICT, PERMISSION, AND IRRELEVANCE

Support is an important concept in phylogenetic inference. We often speak of particular data supporting a phylogenetic hypothesis, and a number of indices are widely used to quantify support (see, e.g., Wilkinson et al., 2003, for a recent discussion). Individual characters can support or conflict with particular relationships in phylogenetic trees, and characters can be treated as corresponding to the trees that they directly support (e.g., Wilkinson, 1998). For example, a parsimony-informative binary character corresponds to, and directly supports, a tree with one internal edge, and a multistate character corresponds to one (ordered) or more (unordered) trees with more than one internal edge (assuming all states are informative). This correspondence underpins the various pseudocharacter matrix representations of trees (Wilkinson et al., 2004). Supertrees are phylogenetic inferences based on the evidence (the support) provided