

# **Biology Curators Group Newsletter**

Title: On the State of Preservation of DNA from Museum Spirit Collections

Author(s): Criscuolo, G.

Source: Criscuolo, G. (1994). On the State of Preservation of DNA from Museum Spirit Collections. *Biology Curators Group Newsletter, Vol 6 No 4*, 39 - 41.

URL: <a href="http://www.natsca.org/article/2402">http://www.natsca.org/article/2402</a>

NatSCA supports open access publication as part of its mission is to promote and support natural science collections. NatSCA uses the Creative Commons Attribution License (CCAL) <u>http://creativecommons.org/licenses/by/2.5/</u> for all works we publish. Under CCAL authors retain ownership of the copyright for their article, but authors allow anyone to download, reuse, reprint, modify, distribute, and/or copy articles in NatSCA publications, so long as the original authors and source are cited.

Cumbria and Argyll as a result of *ad hoc* reintroductions by private individuals.

It is difficult to keep track of such covert efforts and odd occurrences of the species can no longer be instantly dismissed as polecat-ferrets or hybrids. Dr Andrew Kitchener at the Royal Museum of Scotland is assessing the extent of cross-breeding between polecats and feral ferrets based on skull morphometrics, pelage examination and DNA fingerprinting (the latter in collaboration with Dr Huw Griffiths at Leeds University).

A large sample of undamaged polecat, feral polecatferret and hybrid corpses from across Britain are needed if the project is to succeed. Andrew would be grateful for access to any specimen handed in to local museums and can be contacted on 031 225 7534.

Any curators interested in promoting local publicity on the polecat's recovery may apply to the Vincent Wildlife Trust's Polecat Project at 3 Knell Cottages, Harcourt Road, Mathon, Nr Malvern, Worcs WR13 5PG, for a free sample of polecat leaflets and associated artwork.

# PUBLICATIONS

Wilthew, P. 1994. *Bugs, or beating unwanted guests.* SSCR Journal Vol 5 (1). - is an account of the 12th meeting of museum conservation scientists at the V&A last november with outlines of speakers accounts of pest control strategies, monitoring and treatments.

In the USA the ASC has published the report *Guidelines for Institutional Database Policies* the result of its two year study and workshop on data sharing and database ethics. Although the sections an the law relating to data are not likely to be of practical use to BCG members, sections on data sharing agreements, data sharing, transfer policies and the responsibilities of owners and users could be of use in developing models in what is as yet a very poorly developed area here.

Goulet, H. and Huber J. (eds) 1993. *Hymenoptera of the world: an identification guide to families*. Published by Agriculture Canada and available via Books Express, PO Box 10, Saffron Walden, Essex CB11 4EW. (Price approx = 63.35). A fully illustrated, minimal jargon key to all 99 families of Hymenoptera and to the subfamilies for the ichneumonoid wasps and the aculeates.

# **Back Issues**

All parts of BCG Newsletter are available from the editor price  $\pounds 2.50$  incl. GB p&p except the following:

Vol 1 parts 7 and 8 Vol 2 parts 1 and 3-7 inclusive Vol 3 part 1 Vol 6 part 1

# Masochist Needed

All volumes of the BCG Newsletter up to and including

volume four were indexed. There has been no index for volume five thus far because no-one has volunteered (or been volunteered) for what can only be described as the equivalent of hard labour. Would anyone with a particular desire to indulge in this secretive and highly specialist activity which I suspect has been carried out in secret for generations by a band of dedicated disciples please contact the editor?

#### PAPERS FROM THE SPIRIT MEETING OF 25 OCTOBER 1993

The following papers have been submitted by speakers at the highly successful BCG meeting at the NHM last year, including those which for various reasons could not actually make it on the day. They provide a particularly useful compendium of practical knowledge and the editor would like to thank not only the authors for their valuable contributions but also Jane Mee (Ludlow Museum) for her tenacity in eliciting such a complete account of the day for the benefit of BCG members. The presentation of such a mass of useful well for the proposed information bodes metamorphosis of BCG Newsletter into the Biology Curator.

# ON THE STATE OF PRESERVATION OF DNA FROM MUSEUM SPIRIT COLLECTIONS.

Museum collections around the world contain many millions of biological specimens which are preserved in formalin or alcohol. A large number of these specimens were collected at great expense from remote parts of the globe, and would be difficult or impossible to recollect. Some represent extinct species, and others represent species which are threatened to become extinct in a few years from now. The fact that molecular techniques have progressed tremendously in the last few years has widened very much the scope of museum collections, so that they can now be analyzed in different ways and their study can provide invaluable new information.

The molecular data obtained from DNA work is a precious complement to morphological and physiological studies, and sometimes provides a clear answer to problems that cannot be solved with the more traditional morphological approach.

This is the case, for example, with organisms that display a limited variability or a limited number of distinctive characters in their morphology, and are for to discriminate these reasons difficult or classify.Molecular data can also be used, as in the case of the quagga, to establish the phylogenetic position of an extinct animal, or as in the case of rare and endangered species, to make an informed choice on which conservation policies should be adopted to preserve authentic genetic diversity in critical areas of the world. In other cases the use of museum specimens can facilitate the study of organisms that are temporarily uncollectible for reasons of cost, availability, security, politics or geography.

Spirit collections are very heterogeneous and include all collections of biological specimens which have been prepared by an initial fixation of the tissue and then submerged in preservative fluid and stored in a closed vessel. The purpose of fixation is primarily to arrest the physical and chemical changes that would occur upon the death of a tissue. Fixation aims principally to preserve the overall form and appearance of a specimen and also serves the purpose of sterilizing the specimen, by killing any bacteria or fungi that may be present. generally achieved using fixation is True formaldehyde, which can be used as an aqueous or buffered solution known as formalin (Simmons,1991; Pabst, 1987).

Formalin (which may be used in conjunction with other substances like glutaraldehyde or phenol) involves the formation of permanent covalent bonds which link together the molecules that compose a tissue, so that they are unable to undergo rearrangement (Alberch,1985). The fixative serves as the linking agent in this process and it becomes permanently incorporated in the fixed material. In practice, some fixation reactions are not truly irreversible, and dissociation may slowly occur in the absence of excess fixative, or as a result of a fall in pH in the preserving medium, or by treating the specimen with substances able to remove the fixative.

In theory, most fixation procedures are directed at the immobilization of protein, so that in fact nucleic acids and other molecules become fixed almost fortuitously. Nucleic acids also offer some substrate for fixation, as for example amino groups, nitrogenous heterocycles and hydroxyl residues, but some of these are involved in internal hydrogen bonding, and so may not be very reactive (Stoddart, 1989). There are several ways in which nucleic acids can be damaged by fixation. DNA can be generally damaged by the cross linking of the molecules, but there are some more specific sites on the DNA molecule which are susceptible to damage. For example, the cleavage of the CN link between the sugar and the base leads to a loss of bases, while the hydrolysis of the phosphodiester bonds at positions 3' or 5' leads to shorter strands of DNA. Moreover, the bases can undergo oxidation, which will prevent the two complementary strands of the DNA molecule to pair correctly (Eglington and Logan, 1991).

Since DNA from fixed specimens can present any combination of the four problems mentioned above, it is not surprising that the molecular data from museum specimens is often incomplete and fragmented.

Another important class of compounds widely used in the preservation of museum specimens are the pseudofixatives. These compounds are less damaging to DNA, and they are sometimes also used as preservatives. They include ethanol, methanol, industrial methylated spirit (IMS), chloroform, acetone, and acetic acid, and they act by unwinding and disordering protein, and by altering the patterns of hydrogen bonding in the tissues by removing water (Stoddart, 1989). The net result of this process is a sort of macromolecular tangling, mainly composed of protein, but also involving nucleic acids and other molecules. This kind of process causes less damage to the DNA and is usually reversible, especially if high percentage ethanol is used both as fixative and as preservative medium. DNA molecules retrieved from specimens treated in such way are usually in relatively good shape.

According to my own experience in dealing with museum specimens, it is possible to extract viable DNA from the majority of specimens. However, the yield and state of conservation of the DNA are very variable. The fact that very often the procedures of preservation for particular specimens have not been recorded in detail means a lot of guess work for the molecular biologist, who will never be able to predict which specimen will yield viable DNA, and may waste time and resources. When I worked with pickled lizards I had at my disposition a few specimens, which ranged from 50 years old to contemporary, and most of them were known to have been fixed in formalin and preserved in IMS. However, I could not refer to a detailed record of the procedure used, concerning for example any preliminary or intermediate treatment of the animal, time of fixing, or if the formalin had been injected, all of which can make a difference in the state of conservation of the DNA. There are still many things that we do not know about the way in which DNA is affected by preservation procedures, and a detailed record of these procedures could help us to study the link between preservation procedures and molecular damage.

Perhaps the most rewarding museum specimen I have ever come across is a hundred years old lizard which had been simply pickled in cheap brandy by a collector and never touched since. It gave by far the best yield of DNA and no problems when it came to gene amplification by PCR, in spite of being much older than the formalin-fixed lizards (Criscuolo, 1992). This is a good example to illustrate the paradox that alcohol is traditionally considered a very poor fixative, compared to formalin, and yet, the oldest fluid museum specimens, which have survived 300 years were prepared without formaldehyde, which came into use only 100 years ago. It is only very recently that curators have begun to realize that formalin-fixed specimens might not last for as long as it had been thought.

The study of DNA from museum collections is a very recent field, and we are only now beginning to acquire some of the specialized knowledge that will be useful to the next generation of curators and researchers.

It is in this context, that I would like to stress a few important points:

- a) the age of a specimen may affect the state of conservation of the DNA less than the preservation procedures
- b) a detailed record of the preservation procedures helps the molecular biologist tackle some of the problems posed by DNA alteration

- c) there is a need to reconsider some of the preservation procedures on newly collected specimens so that they cause minimal damage to DNA
- d) there are alternative methods of preservation of animal tissue or of entire specimens for DNA work, for example the use of 90% or absolute ethanol, or deep freezing at  $-70^{\circ}$ C.

Molecular biology is a fast evolving field, and new techniques and applications are constantly being devised that are relevant to spirit preserved museum specimens. Many museums around the world now have molecular biology facilities, and *ad hoc* policies are being introduced to regulate the loan and sampling of biological specimens. Curators and molecular biologists are beginning to collaborate towards a new understanding of specimen conservation, and I have no doubts that in the near future all newly collected biological specimens will be preserved with its possible use in molecular biology very much in mind.

#### Acknowledgments

I wish to thank Tim Littlewood of the Natural History Museum for his feedback on the manuscript and his advice on the subtleties of the English language.

#### References

Alberch, P. 1985. *Museum collections: their roles and future in biological research*, pp. 29-42 (E.H.Miller ed.). British Columbia Provincial Museum Occasional Papers Series.

Criscuolo,G. 1992. Ancient DNA Newsletter Vol 1, n.1, pp.12-13. Wayne and Cooper (eds). London. Institute of Zoology, The Zoological Society of London

Eglington,G., Logan,G.A. 1991. Phil.Trans.R.Soc.Lond. 333: 315-328.

Pabst, R. 1987. The Anatomical Record, 219: 109-112.

Simmons, J.E. 1991. *Natural History Museums: Directions for Growth*, pp. 69-89. Cato and Jones (eds), Texas Tech University Press, Lubbock, iv+252pp.

Stoddart, R.W. 1989. "Conservation of Natural History Specimens" Meeting, University of Manchester.

#### Giuli Criscuolo

Department of Zoology, The Natural History Museum

# THE REORGANISATION OF THE NATURAL HISTORY MUSEUM'S AVIAN SPIRIT COLLECTION

#### Introduction

The Natural History Museum's avian spirit collection, currently comprising *circa* 15000 specimens of 3000 species, is the third largest in the world and the one holding the greatest number of species (Zusi *et al* 1982). The collection was moved from London to Tring in 1972 and is now situated on the specially ventilated ground floor of a purpose-built air-conditioned building. Jars of specimens were arranged in family order in accordance with Peters' *Checklist of Birds of the World* (1931-86) and placed on rows of open steel cabinet shelving. Further information on the composition of the collection and on its curation in the mid 1970s may be found in Blandamer and Burton (1979).

Preparation of fresh material had led to considerable expansion of the collection and had created a considerable overcrowding problem. This adversely affected both respiriting and the location of specimens. Fortunately, there remained three empty rows of cabinets set aside for expansion at the end of the collection which could be utilised, and a major reorganisation project for the whole spirit collection was decided upon in 1992.

#### Aims

-To eliminate the existing overcrowding problem, making specimens accessible.

-To shelf-label and index the collection at the generic level to aid efficient location of specimens.

-To locate and separate all extinct and endangered species from the main collection.

# Procedure

Following a preliminary rough assessment of the amount of space each family would require, the rearrangement of jars began. Two staff members working backwards from the end of the collection towards the beginning, arranged the jars for each family in alphabetical order of genus using the free shelving as working space. Once in alphabetical order, the jars were then shifted to their new location at the end of the free shelving and arranged in Peters' order of genus and species. The jars were positioned only 1-2 deep on the shelving to facilitate the reading of specimen labels as well as to assess spirit levels. The bottom shelf of each cabinet was left empty wherever possible to allow for future expansion. Temporary post-it'labels were used to list the contents of each shelf of every cabinet.

At the same time all unlabelled, illegible or clearly misidentified jars of specimens were removed from the collection and set aside for a further two staff members to work on. Once the problems were rectified, these jars were re-incorporated into the newly arranged collections.

Jars which were labelled with obsolete generic names were temporarily given 'post-it' notes with their modern names. When the general reorganisation was complete, permanent internal labels were prepared and added to these jars without removing the original labels. All extinct and endangered material was removed from the main collection and incorporated into a separate extinct and endangered collection which is housed, also in Peters' order, in three locked steel cabinets. Once the rearrangement of the collection was complete, new family labels were printed for each