Care and Conservation of Natural History Collections

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Genetic material

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Introduction

Many conservators are now aware that museum collections are potentially important sources of genetic material. Although it is fanciful to suppose that living animals will ever be resurrected from samples of preserved genetic material, it is nonetheless possible with today's technology to obtain taxonomic information from the genetic material preserved in museum specimens. It is important to appreciate that taxonomic information is not only valuable for studies of extinct or endangered species but also provides the basic data needed for population studies centered on, for example, the impact of environmental changes on animal and plant communities. Even the most mundane specimens should therefore be treated as vital sources of genetic material for future research. A conservator anxious to preserve the genetic material in a museum specimen will ask three questions:

1. How rapidly and in what way does the genetic material degrade after death?
2. What should I do to prevent or at least retard this degradation?
3. Are there any general preservative treatments that I should avoid on the basis that they may promote degradation of the genetic material?

This chapter attempts to answer these questions.

Degradation of the genetic material

The chemical nature of the genetic material

In all organisms except a few viruses the genetic material is DNA (deoxyribonucleic acid). This chemical is a polymer made up of units called nucleotides linked together in an unhranched linear chain. In the living cell a molecule of DNA is made up of two polymers wound around one another in the famous double helix configuration. The polymers are extremely long: each human cell contains twenty-four DNA molecules with an average length of 125 million nucleotides. The most important feature of DNA is that there are four different nucleotides, called A, T, G and C, that differ from one another in subtle chemical ways and can be linked together in any order in a DNA polymer. Genetic information, which the cell uses to direct its metabolic activities, is specified by the order ('sequence') of nucleotides in a DNA polymer. The nucleotide sequence is also the basic information used by the molecular biologist to determine the taxonomic position of the organism. The aim of the conservator must therefore be to preserve a specimen in such a way that the nucleotide sequence of its DNA is not lost.

The types of degradative event that might occur

Preservation of the genetic material in a wholly intact form is an objective that is unattainable at the present time. Any kind of physical or chemical challenge, including the processes thought to occur inside cells immediately after death, will result in structural changes to DNA molecules. We should therefore start by delineating the types of degradative event that might occur and examining the effect that these changes will have on the ability of the molecular biologist to read the nucleotide sequence and hence obtain taxonomic information from the preserved DNA. Broadly speaking, there are four possible types of degradation:

1. **Denaturation** is the technical term for the unwinding of the double helix. The two strands of the helix are held together by relatively weak chemical bonds that are easily disrupted. But the conservator does not need to worry greatly about denaturation as it does not affect the nucleotide sequences of the two polymers in the helix: the individual polymers may become more susceptible to other types of chemical attack after denaturation but in effect the DNA is unchanged.
2. **Cross-linking** can occur between the strands of a double helix so that the two DNA polymers become bonded together. As with denaturation, the nucleotide sequences of the individual DNA polymers are unchanged. However, cross-linking is undesirable as it can interfere with the analytical techniques used to read a nucleotide sequence.
3. **Strand breakage** occurs if the bonds linking adjacent nucleotides in a DNA polymer are broken. A certain amount of strand breakage can be tolerated as taxonomic information can be obtained from very short pieces of DNA. Sequences longer than 1000 nucleotides in length are rarely needed, and even fragments of DNA as short as 250 nucleotides are useful.
4. **Chemical modification** is any type of chemical change that occurs within a nucleotide. The change could involve addition, removal or replacement of a chemical group. Chemical modifications are highly undesirable and should be avoided because their effect is to change the nucleotide sequence. Preserved DNA that has been chemically modified to any great extent is of no value.

Degradation of DNA after the death of an organism

When an organism dies the controls over that cellular and physiological processes existed during life are lost and the organism undergoes autolytic decay. During this period DNA molecules degrade relatively rapidly clue to the effects of enzymes and reactive chemicals that are released in the tissues. Little is known about the precise events that occur during autolysis but it appears that the main form of DNA degradation during this period is strand breakage, with the polymers breaking down over a period of hours into fragments only a few hundred nucleotides in length.

Once the autolytic decay processes end, either naturally or because of artificial treatment, the DNA molecules enter a period of much slower decay caused by environmental factors. The most important of these are hydrolysis, oxidation, radiation and heat (Lindahl, 1993):

1. **Hydrolysis** is promoted by the presence of water. Hydrolysis of DNA results in strand breakages and in chemical modifications to nucleotides (Greer and Zamenhof, 1962; Lindahl and Karlstrom, 1973). However, in most specimens at least some of the DNA which provides a degree of protection against hydrolytic attack (Lindahl and Nyberg, 1972).
2. **Oxidation** is caused by oxygen in the air. Oxidation of DNA results in strand breakages (Epe *et al.*, 1993) and chemical modifications (Lindahl, 1993).
3. **Radiation** effects are more complex. Ultraviolet radiation (e.g. from sunlight) causes chemical modifications in DNA (Bernstein and Bernstein, 1991.) but at ambient levels does not penetrate beyond the surface of a specimen. Cosmic rays are highly penetrative but do not appear to be particularly damaging. Ionising radiation (e.g. radioactive chemicals) can cause

many types of chemical modification, partly due to the generation of oxidants such as hydrogen peroxide (Winyard *et al.,* 1990). However, it is unlikely that a museum specimen will come into contact with ionizing radiation.

4. **Heat** on its own causes DNA denaturation but its most important effect is to speed up all other types of damage (see, for example, Lindahl and Nyberg, 1974).

Prevention of DNA degradation in museum specimens

Two factors need to be taken into account when considering how to preserve the DNA in museum specimens. The first is to define positive steps that can be taken to minimize the autolytic and environmentally induced degradation of DNA that takes place in the specimen. The most important of these positive steps is to reduce as much as possible the time that elapses between the death of the organism and its preservation. Other than that the only general advice that can be given is that the preferred preservative methods are a dried specimen or one preserved in formaldehyde or ethanol, and that the specimen should not be stored above room temperature. These guidelines are summarized in Table 6.1.

If the specimen is representative of an endangered species, or unusual in some other way, then storage of purified DNA should be considered. DNA extraction should preferably be carried out before death (e.g. a blood sample) or immediately after, and the purified DNA stored in solution or as a desiccated powder at low temperature, for instance in liquid nitrogen, under which conditions relatively little damage accumulates. This is not an option for most conservators and if DNA purification is considered desirable then contact should be made with the Institute of Zoology, London or with San Diego Zoo, both of whom have facilities for long-term storage of purified DNA.

Table 6.1 Guidelines for the preservation of the genetic material in museum specimens

Positive steps to preserve **DNA**

1. Make sure that a minimal time elapses between death of the specimen and its preservation. If preservation cannot be carried out immediately then freeze the specimen.
   Aim for a dried specimen or one preserved in formaldehyde or alcohol.
3. Ensure that the specimen is not subjected to temperatures in excess of 30°C, and if possible maintain it at a low room temperature. Avoid direct sunlight Which might cause localized heating in the specimen.

Treatments to avoid

4. Avoid the use of acids or alkalis.
5. Do not use any chemical that is known to be mutagenic or carcinogenic.

Treatments that might promote degradation of DNA

The second factor relevant to DNA preservation is the avoidance of artificial treatments that might actively degrade the DNA in a specimen. Unfortunately, with the exception of formaldehyde fixation (see below under Preservation of DNA in formaldehyde-fixed specimens) and insect preservation (see below under Preservation of insects for DNA studies), there have been virtually no direct studies of the effects of standard conservation treatments on the DNA present in museum specimens. Consequently we must make use of nucleic acid chemistry to identify relevant compounds that might be deleterious. This presents us with several problems, the first being the fact that molecules as elaborate as DNA do not have simple chemistries. Many chemicals react with DNA and in several cases more than one type of alteration can occur. The second problem is that the chemistry of DNA has been studied predominantly in aqueous solutions, and we cannot be sure whether the reactions that occur in water also occur in dry or spirit-inundated tissues. A third problem is that all studies of DNA chemistry have been carried out with pure DNA whereas in the cell DNA is attached to proteins and may display different reactivities, as appears to be the case with its interaction with formaldehyde. Finally, for a chemist a week is a long time to study a chemical reaction, whereas we are interested in reactions occurring over decades if not centuries.

Despite these problems we can make a few generalizations. DNA is attacked by both acids

Table 6.2 Compounds that cause chemical modifications to pure DNA under mild conditions (i.e. at or near physiological pH and ambient temperature) — for details see Brown (1974) and Blackburn (1990)

Chemical type	*Examples*
Aldehydes	Formaldehyde. glyoxal, ninhydrin
Alkylating agents	Dimethyl sulphate. alkyl halides
Ammonium derivatives	Hydroxylamine, hydrazine, semicarbazide
Aromatic nitrogen compounds	Aromatic amines, azo-dyes
Bisulphites	Sodium bisulphite
Borohydrides	Sodium borohydride
Carbodiimides	*N,N*- dicyclohexylcarbodiimide
Halogens	Bromine, iodine, iodine chloride
Mercurics	Mercury (II) acetate, mercury (II) chloride
Nitrites	Sodium nitrite, nitrous acid
N-Nitroso compounds	Nitrosourea
Oxidizing agents	Potassium permanganate. osmium tetroxide. peracids. hydrogen peroxide

and alkalis, the former causing drastic chemical modifications that will make the nucleotide sequence unreadable. Alkalis promote strand breakage, this occurring readily with *0.5* M sodium hydroxide (Blackburn, 1990). The acid and alkali effects are complicated by the presence of other chemicals. Metal ions such as La^{3+} and $C^{3+'}$ catalyse strand breakage, and others, including Ca^{2+}, Zn^{2+}, Ba^{2+} and Pb^{2+} are thought to have similar effects (Huff *et al.,* 1964). The concentrations required are very low: for example, 1 mM Pb^{2+} at physiological pH is active. Other ions, such as Mg^{2+}, inhibit strand breakage. Various other compounds that are implicated in strand breakage of DNA are osmium tetroxide (Burton, 1967), potassium permanganate (Ackman *et al.,* 1990) and possibly hydroxylamine plus acid (Kochetkov *et al.,* 1967). The diversity of these compounds indicates that there are probably many others with similar effects.

Other types of chemical modification are caused by a bewildering variety *of* compounds, but many *of* these compounds are mutagenic or carcinogenic and so probably not among the conservator's favourite chemicals, though some may crop up in preparations used to prevent microbial and insect infestations. Mutagens often act by adding alkyl groups (e.g. —CH) to nucleotides which, as well as being a significant chemical modification, can also lead to strand breakage or cross-linking (Lawley. 1966). A list of compounds (including several not recognized as mutagenic) that are known to cause chemi-

Table 6.3 Predicted effects on DNA of compounds relevant to preservation of museum specimens.

Compounds that are probably safe	*Compounds that are probably not safe*
Acetone	Ammonium hydroxide
Alcohols	Carbon tetrachloride
Alum	Chioropicrin
Arsenicals	Chromic acid
Bendiocarb	Citric acid
Benzene	Dichlorovos
Borax	Ethylene dichloride
Camphor	Glutaraldehyde
Carbolic acid (phenol)	Lead salts
Carbon disulphide	Lindane
Chloroform	Mercuric salts
Chromates	Methyl bromide
Dioxane	Organomercuric salts
Ether	Organophosphates
Ethylene oxide	Paradichlorobenzene
Gasoline	Pentachlorophenol
Glycerine	Perchloroethylene
Glycerol	Sodium silicofluoride
Hydrogen cyanide	Sulphur fluoride
Lysol	
Magnesium carbonate	
Naphtha	
Naphthalene	
Phosphine	
Potash	
Potassium nitrite	
Potassium phosphate	
Propoxur	
Salt (sodium chloride)	
Sodium acetate	
Sodium bicarbonate	
Sodium dithionite	
Sodium phosphate	

cal modifications to DNA under relatively mild conditions is given in Table 6.2.

Finally, Table 6.3 deals with the possible effects on DNA of some of the compounds relevant to preservation of museum specimens. Those chemicals that are known to react with DNA should clearly be avoided, though some are probably not as deleterious as they might initially appear.

Preservation of DNA in formaldehyde-fixed specimens

One preservative technique that has been studied specifically with reference to DNA is formaldehyde fixation. Formaldehyde is deleterious to pure DNA as it causes rapid denaturation followed by strand breakage and also has the capacity to cause chemical modifications (Vachot and Monnerot, 1996). However, in the cell DNA is attached to proteins and this modifies the action of formaldehyde, the main reaction now being the formation of cross-links between protein and DNA (Koshiba *et at,* 1993) and under these circumstances there appears to be little, if any, substantial damage to the DNA polymers themselves (Haselkorn and Doty, 1961). In fact, the main problem with formaldehyde-fixed specimens is probably not the action of formaldehyde but the possibility that the formaldehyde oxidizes to formic acid (Taylor, 1977) which, like all acids, will cause chemical modifications to DNA.

Vachot and Monnerot (1996) compared the effects of a number of different formaldehyde fixation protocols on preservation of DNA in amphibian muscle tissue. They found that with unbuffered 10% formaldehyde a low fixation temperature (4°C) was critical for DNA preservation, which is impractical for specimens collected in the field. However, fixation in buffered formaldehyde (e.g. 10% phosphate-buffered formaldehyde, pH 7.4) was effective in preserving DNA regardless of the fixation temperature. The presence of 1 M (3-mercaptoethanol had some beneficial effects on DNA retention, as did the addition of 6 M guanidine, though the latter is not recommended because of the toxicity of guanidine and because it causes changes in tissue texture. Unbuffered formaldehyde plus 173 mM NaCl was better than salt-free unbuffered formaldehyde but not as good as buffered solutions. For the best combination of DNA preservation and tissue morphology the authors recommended 10% phosphate-buffered formaldehyde, pH 7.4 + 1 M R-mercaptoethanol, with fixation at room temperature for not more than three days.

Preservation of insects for DNA studies

Although not specifically directed at museum conservation, the study by Post *et of.* (1993) of techniques that are compatible with future analysis of DNA in preserved insects is worth summarizing. Their main aim was to identify methods for preserving insects between collection in the field and examination in the laboratory, the test material being the dipteran *Simulium damnosum.* An important result was that pinned insects collected eight years previously gave undetectable yields of DNA, as did specimens fixed in 10% formal saline and stored for one year. These methods should clearly be avoided if DNA preservation is hoped for. The highest yields of DNA were obtained from flies preserved in liquid nitrogen, which is inappropriate for museum collections, but good DNA yields were also obtained from specimens fixed in 60%, 80% or absolute ethanol and stored at 4°C. Another method promoting DNA preservation was drying over silica gel: flies were placed in a small glass bottle packed at the bottom with silica gel and put in a warm place (e.g. in sunlight) until dried, and then stored over silica gel at room temperature or at 4°C. In contrast, fixations in Carnoy's solution, methanol or propanol were all less successful with regards to DNA preservation.

Conclusions

It is still too early to give definite guidelines for ensuring that DNA is preserved in museum specimens. Only with formaldehyde fixation and insect preservation has there been any systematic comparison of alternative methods, and our knowledge of DNA chemistry, especially of cellular DNA as opposed to pure DNA, is too patchy to enable the possible effects of all relevant compounds and treatments to be

predicted. The one encouragement for the conservator is the fact that DNA is routinely retained for at least 5000 years in naturally preserved bones, teeth and plant remains (Brown and Brown, 1994), and is present in existing museum specimens preserved with no consideration given to the genetic material (see, for example, Higuchi *et al.*, 1984). This is not an excuse for ignoring positive steps to preserve DNA, but it does suggest that the standard conservation procedures are adequate even if they are not perfect.

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