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The Impact Of Frozen Tissue And Molecular Collections On Natural History Museum Collections

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This was a dissertation submitted in partial fulfilment of the requirements for the degree of MA in Museum Studies of the University of London in 2003

Abstract
This paper examines the impact of frozen tissue and molecular collections on traditional natural history museum collections. This was carried out by an examination of current literature, by talking to people who work with frozen tissue and molecular collections and by input of my own views as a curator of Lepidoptera (butterflies and moths) at The Natural History Museum, London. The first part deals with definitions of natural history museum collections and what they are used for. Then there is an examination of the issues surrounding the storage facilities for tissues and molecules, deposition of molecular material and the suitability of current and future museum specimens for molecular work. A large part of the future of systematics and taxonomy lie with molecular studies. As one of the main purposes of natural history museums is the study of systematics and taxonomy then natural history museums have to embrace frozen tissue and molecular collections to survive. I conclude that molecular collections are an important part of the future of natural history collections.

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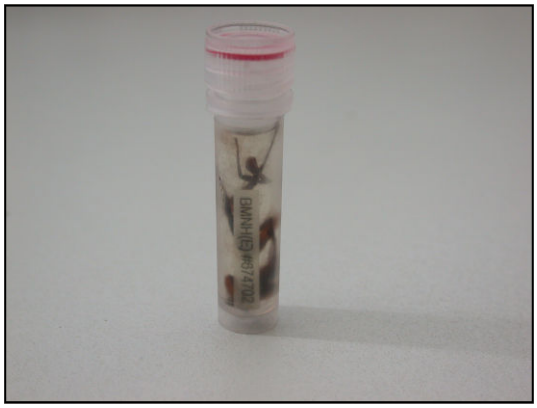

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CHAPTER 1 - Introduction to natural history museums and their collections

"Curators often see molecular biologists as sort of evil interlopers who soak up valuable resources and take up space that could be better used for storing collections. Some of the molecular biologists see curators as traditionalists who don't recognise or are incapable of recognising the path-breaking importance of their research." (Thomas, 2000)

1.1 Introduction

In the past 30 years the use of molecular techniques to answer questions of systematics and taxonomy has exploded. Although morphological studies have not been dismissed, the study of systematics increasingly relies on molecular data to confirm or challenge morphological studies. As natural history museums have traditionally been a depository for specimens and conducted systematic and taxonomic research into these specimens, it is reasonable that they are also going to embrace molecular studies. Some curators view these developments with suspicion particularly as many molecular studies cannot use traditional museum specimens for a number of reasons, generally due to the degradation of molecules over time. Although some molecules have been extracted from museum specimens, most molecular workers prefer fresh material and in some cases can only use fresh material. These frozen tissues and molecules also present unique storage problems. The use of freezers, cryogenics and molecular extraction equipment present museums with a further increase on ever tightening budgets. I will examine a number of issues concerning frozen tissue and molecular collections and their impact on natural history museum collections. These issues only affect the large national or university museums with encyclopaedic collections and staff that carry out research into taxonomy and systematics. The smaller local museum is not directly affected by these issues but may be affected if governmental resources are redirected to the larger national museums.

1.2 What are natural history museums and what is their purpose?

Natural history museums have been in existence for 250 years with the first natural history museum, the British Museum, opening in 1753 (Stearn, 1998:9). Other museums soon opened across Europe such as the Muséum national d'Histoire naturelle, Paris, founded in 1793 (Muséum national d'Histoire naturelle, 2003). In the following century natural history museums started to open in the USA. The Smithsonian (or National Museum of Natural History) in Washington D.C. opened in 1846 (Smithsonian Institute Libraries Online Exhibition, [No Date]) and the American Museum of Natural History, New York opened in 1869 (Kolb, 1999). These are probably the four most important natural history museums in the world due to the large encyclopaedic collections they house which includes a large proportion of the world's type material (see below for definition of type material). Many academic institutes also have natural history museums associated with their campus such as Oxford University Museum, England and Harvard Museum of Natural History, U.S.A.

Aside from public education and entertainment, natural history museums other role is the safeguarding of collections of specimens. These specimens are used in the study of taxonomy (i.e. scientific description and naming of living and fossil organisms; placing them within a system of classification; and developing systems for identification) and in the study of systematics (i.e. the naming and investigation of the characteristics and relationships of organisms), (The Natural History Museum, 2003a). Natural history museums are also depositories for type specimens. A type specimen is the unique representative of its particular species

and the specimen to which the scientific name is correlated, once defined a type specimen remains forever of key value, and will be required for study whenever that species comes under review (Thackray & Press, 2001:70).

1.2.1 Taxonomy, systematics and molecular biology

Taxonomy is based on the binomial system of classification first applied in 1758 when Carl Linnaeus published the tenth edition of *Systema naturae* (Koerner, 1999:199). However this was not totally accepted across the scientific community until 1867 in the case of plants and 1905 in the case of animals (Koerner, 1999:201). Systematics investigates the relationships between individuals, populations and species. One of the key tools for investigating systematics is that of phylogenetics. Phylogenetics attempts to recover genealogical relationships among groups of organisms and produces classifications that exactly reflect those genealogical relationships (Wiley, 1981:6). From this phylogenetic trees are produced which utilise hypotheses of character transformation to group taxa hierarchically into nested sets and then interpret these relationships (Kitching *et al.*, 1998:213).

Taxonomy and systematics were traditionally based on morphological characters to separate and classify organisms. Since the 1960's biological research including taxonomy and systematics has moved with ever increasing speed towards the molecular level (Dessauer & Hafner, 1984:1). The molecular level includes examination of proteins, DNA (deoxyribonucleic acid), and RNA (ribonucleic acid), although the vast majority of research at the present time involves DNA. The study of DNA really accelerated in the 1980's when the Polymerase Chain Reaction (PCR) was invented by Kary Mullis in 1983 (Mullis, 2002a). Mullis was awarded the Nobel Prize for Chemistry in 1993 for the invention of PCR (Mullis, 2002b). PCR enabled tiny fragments of DNA to be copied millions of times, cheaply, easily and in only a few hours. Tiny amounts of DNA could now be taken from a hair, a feather, blood or a museum specimen and amplified many times until useable quantities for study were obtained. PCR also revolutionised many other disciplines such as forensic science and medicine. Certainly systematics today relies heavily on molecular information to ascertain the relationships between species. Molecular information has provided many more characters for which the systematists can now use to build molecular phylogenies. Taxonomy generally does not make full use of molecular information at present as most species descriptions are still based on morphological characters. Although there is much heated debate as to whether this should remain the case (for more on this issue which is beyond the scope of this paper see Lipscomb *et al*., 2003; Seberg *et al*., 2003; Tautz *et al*., 2003).

Most major natural history museum collections are laid out systematically being sorted by order, family, genus and species. Without some kind of systematic system and some way of retrieving this information (i.e. a database, electronic or otherwise) then the collection would be unworkable. At the present time the systematic layout of most collections is usually based on morphological characters.

1.2.2 What are natural history collections?

Traditionally natural history collections consist of specimens preserved broadly in two ways: dry (i.e. pressed, pinned, boxed or mounted) or wet (i.e. preserved in alcohol or formalin), (Anon, 1998). Natural history collections tend to be divided into botanical and zoological collections.
For the most part botanical collections (which will include Fungi) consist of dried specimens (including seeds) on herbarium sheets and these sheets are ideally stored in pest proof steel or wooden cabinets (see Plate I). There will be collections of smaller specimens such as diatoms, microalgae and pollen which will be mounted on microscope slides. There will also be small amounts of plant material stored in spirit, usually 80% IMS (Industrial Methylated Spirit).

Zoological collections are usually divided into Vertebrates and Invertebrates. Vertebrate collections will include dried skins, skeletons and a large number of whole specimens in spirit, usually in formalin or 80% IMS (see Plate II). Invertebrates collections will include a large number of dried specimens such as pinned insect and shells (see Plate III). Very small specimens such as greenfly will be mounted on microscope slides (see Plate IV). Softer bodied invertebrates that do not dry well such as Jellyfish will be kept in spirit, again usually formalin or 80% IMS. The collection area will mostly contain collections storage furniture with areas set aside for research and curation of the collection. Most museums will also have extensive libraries associated with the collections.

1.3 What are frozen tissue and molecular collections

These collections consist of frozen tissues and molecules separated from specimens. Frozen tissues may include a whole organism (common in the case of insects, less common for larger vertebrates), parts of an organism (e.g. hair or feathers from vertebrates, legs from insects) and may include venom and blood samples. Molecules preserved will include extracted proteins, DNA, and RNA. It is usually the case that frozen tissues are collected first and then when time permits molecules are extracted from these tissues in the laboratory. The tissues and molecules will be stored in a variety of ways but generally in some kind of sealed tube (see Plate V), which will be placed in rack system (see Plate VI & VII). Within the tube the tissues or molecules may be kept dry, stored in 100% ethanol or stored with a buffer. All effort is made to prevent the degradation of the tissue/molecule concerned. The tubes containing the tissues or molecules are then stored in either cryovats which contain liquid nitrogen at –155ºC (see Plate VIII) or in a -20ºC or -80ºC freezer (see Plate IX & X).

Museums with large research collections have had to respond and adapt to molecular research. This has meant the building of molecular laboratories and molecular storage facilities within museums and the employment of specialised staff to conduct this research.

1.3.1 Examples of frozen tissue and molecular collections with facts and figures

The minimum requirement for a frozen tissue and molecular collection is a wet and a dry laboratory and a freezer or cryovat storage area. The wet laboratory will include equipment such as PCR machines, centrifuges, laminar flow hoods (similar to a fume cupboard but creates a sterile environment thereby avoiding contamination of isolated DNA) and a range of chemicals, glassware and other associated equipment. The dry laboratory would contain computer equipment associated with the collection inventory databases and would also be involved in the analyses of the molecular work carried out.

I will take the example of the molecular laboratory of the Entomology department at The Natural History Museum, London as a typical example of a museum molecular laboratory that does not utilise liquid nitrogen for storage of specimens. Facts and figures are supplied by Shazia Mahamdallie, the laboratory manager, Mike Fitton, entomology collections manager and Lejla Buza, assistant laboratory manager. The laboratory was started in 1989 and occupies space that was previously taken up by traditional natural history collections. The area covers approximately 250m^2 out of 6000m^2 for the entire entomology department, plus other office space required by researchers. Twenty staff are associated with the laboratory out of a total of 100 staff in the entire department. Only three of these staff have permanent contracts and the other 17 are a mixture of fixed term contracts, PhD and MSc students. Only permanent members of staff are funded by the museum, the rest of the staff are externally funded. The laboratory consists of two offices, a dry laboratory where the computer work is carried out, five wet laboratory which house four -20ºC freezers, two -80ºC freezers, five PCR machines, two lamina flow hoods and a whole hosts other associated laboratory equipment. The running costs of the laboratory in 2002/3 where in the region of £10,000 which £6,500 of this was spent on service contracts and equipment repairs and £3,500 on laboratory consumables. The approximate costs of equipment are as follows; -80ºC freezers cost £8,000 each, -20ºC freezers cost £2000 each, PCR machines £6000 each and the lamina flow hoods cost £4000 each. Around 20,000 specimens are stored here and they are accessed via the Freezerworks™ database, which cost £5,500 plus helpline/service contract. This approximates to start up costs of £67,500, and running costs of £10,000 per annum. Staffing and computer costs are not included.

The role model for all museum frozen tissue and molecular collections must be the Ambrose Monell Cryo Collection (AM-CC) at the American Museum of Natural History in New York, U.S.A. The collections at the AM-CC are stored in cryogenic vats, which contain liquid nitrogen that keeps the collection at around –155ºC (see Plate VIII). There are eight cryogenic vats in the storage area with space for another four; each cryovat contains enough space for 70,000 samples that are contained in racks (see Plate VII) (American Museum of Natural History, 2003a). There is potential at this facility to store nearly a million specimens. The specimens are fully databased using Freezerworks™ and this database is linked to the morphological collection in the main museum and is online (see http://research.amnh.org/amcc/database/) with images of the morphological specimen (American Museum of Natural History, 2002). The facility is fully alarmed in case of problems with the cryogenic vats or the atmospheric oxygen of the facility (American Museum of Natural History, 2003a). Wilkinson & Huxley (2002) report that it would cost in the region of £500,000 to set up and running costs (including staff) would be in the region of £200,000 per annum. The breakdown of

costs is as follows (from Wilkinson & Huxley, 2002). Infrastructure: Bulk tank (3,000 gallon) £30,000; Cryogenic vats (8 x £20,000) £160,000; Vacuum piping £60,000; Monitoring systems £20,000; Wet lab equipment, £80,000; Database /computer equipment £30,000. The recurrent costs for consumables (liquid nitrogen, cryotubes, etc.) are approximately £25,000 per annum. Overall running costs including staff costs are approximately £200,000 per annum. Building costs would of course be extra on top of this.

Although it is difficult to make exact comparisons if one compares number of specimens held by the Department of Entomology's Laboratory at The Natural History Museum (20,000 specimens, start up cost £67,500) and the AM-CC collection, (up to 1,000,000 specimens, start up cost £500,000), it is clear to see that a liquid nitrogen facility is much more cost effective. Although this is a considerable amount of money to find, molecular biology tends to have far more potential to attract sponsors than traditional natural history collections and grants have been obtained in the past for similar such projects from biotechnology and biomedical companies such as Wellcome or GlaxoSmithKleine. Without external funding it would seem unlikely that a museum is going to have the resources to run a molecular collection facility. One option for The Natural History Museum, London, would be to build this facility as part of Darwin Centre II, which is due to open in 2007 (Wilkinson & Huxley, 2002). It is the running costs of the molecular laboratory that causes most concern. Certainly grants are available to purchase capital equipment but rarely are running costs such as maintenance and replacement of equipment factored into these grants. There are conceivably a lot of problems ahead if running costs are not included and just to keep a molecular facility running may incur budget cuts on other parts of the museum particularly the traditional collection.

1.3.2 A short history of frozen tissue and molecular collections

It was the wide adaptation of protein electrophoresis in the 1960's and 70's that provided the impetus for assembling frozen tissue collections (Engstrom *et al*., 1999:316). The number of comparative molecular papers using collections and published in the Journal of Mammology increased from zero in 1954 to two in 1964, six in 1974, 14 in both 1984 and 1994, and 15 in 1996 (Engstrom *et al*., 1999:316). . As outlined earlier it was the invention of PCR that really accelerated museums into building molecular laboratories. Dessauer & Hafner published the first list of molecular collections in 1984. The list comprises of 87 institutes (56 of which are in the USA). Eight of these institutes are museums (7 in the US and one in Australia) virtually all the rest are universities. Dessauer *et al.* (1996:42) provides the most recent list of institutes holding molecular collections. Here there are 60 institutes listed with 45 in the U.S.A and 14 of these institutes are museums. Universities are certainly carrying out a large portion of current molecular work. At the present time most large museums that carry out taxonomic and systematic research now have associated molecular laboratories and many will have frozen tissue and or molecular collections. The first museum to have a frozen tissue and molecular collections was the Louisiana State University Museum of Natural Science Collection of Genetic Resources which opened in 1978 (Brown, 1999a).

1.3.3 The conflict between curator and molecular biologist

Some curators hold the view that molecular biology will be the death of traditional morphological collections. It is easy to see why this view may be held. Any large museum has seen large amounts of funding being made available in the past 20 years available for the building of molecular laboratories and tissue collections with the perceived neglect of the traditional collections. They have also witnessed specimens being taken from morphological collections for molecular work and subsequently destroyed with no useful information being gained. There has definitely been a lack of understanding of the techniques involved in molecular work and the use they have for taxonomy and systematics. Molecular workers are also as guilty of not explaining their motives or work to curators and morphological workers. They also may have little understanding of a large collection, the history behind it and the issues concerning maintenance of a large collection. Many curators complain that molecular work does not make full use of the collection, that molecular work can be carried out anywhere and that the molecular worker is parasitising the museum. However molecular work relies on using large systematic collections for identifications of specimens to be used in molecular work. Even though there is a large body of evidence as to the best way to preserve new specimens for molecular work, some curators are choosing to still preserve new material in ways they know will not preserve molecular data. Molecular workers are also guilty of disposal of specimens once their work has been completed which could be incorporated in a traditional museum collection. Molecular workers also note that there is a great deal of prestige of having a laboratory associated with a long-standing, well-established natural history museum. Indeed the museum prestige itself can also benefit by having a molecular laboratory and collection. Better communication is needed between the curator, morphological worker

and molecular worker to overcome these problems. Certainly collaborative field trips where specimens for both morphological and molecular work are collected and results jointly published are a good way of encouraging team building. I can imagine that many of these problems will be overcome in the next 10 years or so as the 'old school' retire and the importance of molecular work is fully realised as of benefit to the museum as a whole.

CHAPTER 2- Museum specimens and DNA

2.1 Use of DNA from museum specimens

Museum specimens offer unique opportunities for studying DNA. Nowhere else can the researcher utilise diverse collections of correctly identified specimens going back over 200 years, and in the case of specimens preserved in amber several million years old. Museum collections also offer the chance to study extinct species, rare species or species difficult to collect because of current political situations in their countries of origin. Museum specimens are often extremely well documented and come with full ecological and phenological data.

The demand for access to natural history collections for the purpose of DNA extraction has soared in recent years. A search for the term "Ancient DNA" on the BIOSIS Biological Abstracts database (BIOSIS, 2003) returned no hits for 1980-1984, 2 hits for 1985-1989, 27 hits for 1990-1994, 48 hits for 1995-1999 and 100 hits for 2000-2003. Clearly an exponential increase in the number of papers dealing with ancient DNA, although admittedly not all of these will be relevant to natural history museum collections.

2.1.1 Is DNA still viable in museum specimens?

There are several problems with DNA from museum specimens. The DNA maybe too degraded for analysis due to the original preparation method or subsequent preservation method. The specimen may have also been contaminated with DNA from another organism thereby giving false results. Unfortunately it is impossible to tell this from a specimen until destructive sampling and subsequent analysis has taken place.

2.1.2 Destructive sampling

Molecular techniques result in the destruction of part or even the whole specimen (this is known as destructive sampling). This is nothing new as morphologists often need to employ destructive techniques to see the structures they are interested in (Thomas, 1994a). Large specimens such as mammals may only need a few hairs or an extract of bone to provide enough DNA for analysis. Smaller specimens such as insects will need by comparison a much larger fraction of the specimens although with some groups e.g. moths it may be possible just to use a leg. Some specimens are very small indeed and the whole specimen may need to be used to extract enough viable DNA for study. Certainly the destruction of a whole specimen should be discouraged. In the case of type material, extinct or endangered species careful consideration by the curator is needed before material can be used and if it is the case that the specimen will be completely destroyed then I believe that permission should be refused. Perhaps the worst example of destructive sampling that led to no recoverable DNA and the loss of the specimens concern specimens that are preserved inside amber. There have been various claims of extracting DNA from amber preserved insects many of millions of years old cited by Austin *et al*., (1997). But he failed to detect any authentic insect DNA. They attempted to extract DNA from 15 specimens and failed, they also report on the attempts of three other groups who also failed to produce any DNA. They also identified three cases were the samples where contaminated by DNA from living organisms and also point out that all the samples in question where destroyed during the extraction process. In conclusion Austin *et al*., (*ibid.*) state that due to the lack of significant biological questions addressed by molecular studies, the primary value of amber preserved fossils lies in their excellent morphological preservation and not in the fragmented remains of any DNA whose existence remains speculative at least. This paper reinforces the care need in allowing specimens to be destructively sampled, as in this case specimens were totally destroyed yet no DNA was extracted.

2.1.3 Loan of materials for molecular work

Natural history museums have had a long tradition of lending specimens for morphological work. Recently there has been a demand for specimens to be loaned for molecular work. With this increase in demand for

specimens for molecular work pressure has been put on the curators' time and their ability to judge potential users of the collections as to their ability to extract DNA, causing the minimum of damage to the specimen and maximum benefit to the collection. Pääbo *et al*. (1992) was the first to address the issue of loan material for DNA extraction and gives four criteria to be considered before loan material for DNA work that involves destructive sampling.

- The project must be of sufficient interest to justify destruction of specimens
- The technical competence of the researcher and their laboratory
- Evidence that the specimens involved cannot have DNA sampled from wild populations
- The amount of staff time required to evaluate and process the loan

The first point to be considered is there sufficient justification to damage the specimen in the pursuit of DNA. The technical feasibility of the project should be evaluated independently by molecular biologists. As many of the larger museums already have molecular laboratories it may be prudent to ensure that work is done in the museums own molecular and that an appropriate charge be levied for this.

The second point to be considered is the competence of the laboratory and staff of the institute requiring the material. Do they have a published record of DNA work with museum specimens using small amounts of DNA? If not then the museum should err on the side of caution and lend fewer examples of the material required or insist that the museums own facilities are used. Once competence has been demonstrated then further loans can then be issued the researcher needs to demonstrate that the samples required cannot be obtained from the wild.

For many researchers museums may be a convenient and inexpensive way of obtaining DNA samples. One can imagine a group of closely related species that have a wide geographical distribution. The cost of visiting all these countries that the species occur in and gaining permission to sample DNA from the specimens would seriously prohibit the research concerned. In many cases the museum will represent the only easily available source of DNA. However when the cost of curation and storage of DNA samples is taken into consideration, the museum cannot be seen as the cheap option and samples from living populations may well suit the researcher better.

2.1.4 What should happen to extracted molecules?

The question of what to do with the extracted DNA also arises. With any DNA extracted from museum specimens the results are published and for some researchers this is deemed enough. The extracted DNA or aliquot has in the past been disposed of or held onto by the researcher. Whitfield & Cameron (1994) argue that many museums do not have the facilities, budget or curatorial skills to store tissues or molecules over a long period of time and that museums should not require the borrower to return material, claiming that the borrower could look after the sample better than most museums. However actions like this could lead to samples of museum material being scattered amongst academic institutes and there is no guarantee that they could look after the material any better than a museum. In a reply to Whitfield & Cameron (1994), Hafner (1994) points out that many samples have been lost by poor curation on the part of researchers, and that the emphasis on preservation of data rather than samples will only perpetuate this wasteful practice. Hafner (*ibid*.) also points out that there are many institutes such as the Louisiana State University Museum that will accept tissues and molecules for long-term storage. With this in mind it is irresponsible of researchers to waste material. The problem of disposal of extracted material is highlighted by Pääbo *et al*. (1992), where species of high interest to researchers such as Galapagos Finches may receive many requests for samples for molecular work. This is only a finite resource so Pääbo *et al*. (1992) argue that returned samples of molecular extracts should be lent out rather than destroying parts of or whole specimens for identical pieces of research. Pääbo *et al*. (*ibid*.) also suggest that where practical DNA from wild populations should be used in preference to museum specimens. This may not be possible in a number of cases particularly where the species required is rare, endangered or even extinct. Certainly botanists probably have an easier time obtaining samples from wild caught populations as many botanical gardens often contain a wide variety of plants including endangered species. Although zoos do contain many species there is a bias towards vertebrates and although some zoos do keep invertebrates the species range in minute when compared to museum collections.

As well as returning the sample back to the museum or depositing it in a suitable repository, each sample needs to be given a GenBank® accession number. GenBank® was set up in 1988 by the National Centre for

Biotechnology Information (NCBI) in the U.S.A., and is a database of nucleotide sequences from more than 130,000 organisms (National Centre for Biotechnology Information, 2003). The majority of journals now require a GenBank® accession number before an article may be published and GenBank® will also include details of the repository where the sample is held (National Centre for Biotechnology Information, *ibid*.). It is also linked to two other large databases of molecular information, DDBJ (DNA Data Bank of Japan), and EMBL (European Molecular Biology Laboratory) (National Centre for Biotechnology Information, *ibid*.). As the GenBank® database is the central depository for molecular sequence information it is essential that any samples lent to researchers by museums must have a GenBank® accession number associated with them.

CHAPTER 3 - Preservation techniques and molecules

3.1 The effect of preservation techniques on molecules

Many museums still have an active collecting policy, particularly with invertebrates as there is still much to be learnt about systematics and many groups of invertebrates are poorly known and many species are still awaiting description. There is plenty of evidence that many museum specimens are totally unsuitable for DNA extraction. This is the due to treatments both physical and chemical before and after preservation.

3.1.1 Chemicals used in preservation for morphological studies and their affect on DNA

Chemical treatments that are good for morphological work can be extremely detrimental to molecules required for molecular work and vice versa. There is also often no documentation associated with specimens as to which chemical and physical treatments the specimen has been subjected to. Pre molecular times, understandably nobody gave a thought to the effect of processes on molecular structure and no documentation was kept with the specimen. There are many chemicals in use that may affect DNA. Lists of certain chemicals that affect DNA are given by Brown (1999a: 136) and Carter (2002). Their lists are not comprehensive and the list provided by Brown (*ibid*.) is divided into 'probably safe' and 'probably not safe'. I will consider the following three chemicals in more detail, ethyl acetate (used for killing insects), 80% IMS (used for killing and preserving invertebrates and preserving invertebrates) and formalin (used for fixation and preservation of both invertebrates and vertebrates). These are chemicals commonly used in the preservation of museum specimens. Zoological specimens have to be killed before they can be preserved. The method of killing varies from group to group, although whole vertebrates except fish are rarely collected today. A large amount of insect collecting still occurs as museums play a major role in the naming and classifying of insects. Although there are many methods for killing insects for museum collections, traditionally most insects were either killed by gassing with cyanide (cyanide is no longer used as it is banned in most countries) or ethyl acetate (which is still in use today) and the specimens subsequently air-dried. This method is fine for morphological work but Quicke *et al.* (1999) has shown that method of killing plays a crucial role in the ability to extract DNA. The use of ethyl acetate as a killing agent is extremely detrimental to DNA (Reiss *et al*., 1995; Dillon *et al*., 1996; Quicke *et al.*, 1999). These authors found either little or no DNA from specimens that had been killed with ethyl acetate. Although ethanol appears to preserve DNA well, most of the spirit collection in The Natural History Museum, London is preserved in 80% IMS which is 96% ethanol with 4% methyl alcohol added and then diluted with 20% distilled water. 80% IMS was used in preference to ethanol as ethanol attracts a much higher rate of duty and is therefore more expensive to buy. There is a question as to whether DNA is affected by 80% IMS. Carter (2002) states that 80% IMS gives fair to good results when extracting DNA from invertebrate specimens where as Wilkinson (2001) suggests that storage of specimens in 80% IMS causes further degradation of DNA and that The Natural History Museum should abandon the use of 80% IMS and any new collection should be stored in pure ethanol. Formalin (generic name for a solution containing Formaldehyde of varying concentrations) was commonly used for the preservation of whole animals particularly vertebrates and it is still used today for 'fixing' specimens before they are preserved in ethanol. There appears to be some debate as to whether this affects specimens or not. It is difficult to ascertain the exact chemical nature of formalin, Brown (1999b: 137), Reid (2000) and Carter (2002) state that formalin is very detrimental to DNA. Although it is not the formalin itself but the possibility that formalin oxidises to formic acid, which causes damage to DNA. Vachot & Monnerot (1996) conclude that buffered formalin solutions when the correct chemical composition is used cannot react with DNA.

Many smaller museum specimens are mounted onto microscope slides. I will take the example of Hemip-

tera (true bugs). The specimen is first boiled with potassium hydroxide to remove all fleshy parts leaving only the exoskeleton. The exoskeleton is fixed using alcohol or some other fixative. The specimen is then placed in a mountant (such as euparal or canada balsam), which is placed on the microscope slide. The specimen is placed within the mountant, a coverslip placed on top and the mountant is allowed to set hard. Although the specimen may be recovered from the slide by dissolving out the mountant. Large numbers of specimens, including type specimens, are stored in this way. I can find no reference as to whether slide mounted specimens have viable DNA for study although one would expect not after this kind of treatment.

Botanical specimens are also chemically treated for preservation and this has also hindered DNA extraction. Certainly the use of mercury salts (and arsenic) was commonplace on herbarium specimens to deter fugal growth and pest attack. Not only does this represent a considerable hazard to human health (Rader & Ison, 1999:354) but also can adversely affect DNA as it is listed as 'probably not safe' by Brown (1999b: 136). Although at least with botanical specimens they are ways of detecting whether the specimen has been treated with mercury salts (Rader & Ison, *ibid*.). Jansen *et al*. (1999) conclude that chemical treatment should be avoided if the plant material is to be used subsequently for molecular analysis.

3.1.2 Physical treatments that may affect DNA

Physical treatments affecting specimens include drying, heating and freezing. It has been pointed out by Quicke *et al*., (1999) that rapid drying is essential for preservation of DNA and that this may be the reason why insects specimens killed with ethyl acetate and then subject to slow drying yield no usable DNA. Slow drying of specimens may also lead to fungal growth and Quicke *et al*., (1999) have actually sequenced only fungal DNA from wasp specimens. Contamination of DNA is a major problem in museum specimens. Contamination can come from a variety of sources. Handling of specimens can lead to contamination by human DNA. Often specimens are stored very close to each other, particularly closely related specimens. Thomas (1994b: 316) suggests that replicate extractions should be carried out to test for and ensure contamination has not taken place.

3.1.3 Preservation methods that conserve DNA

Certainly the collecting methods of natural history specimens are now going to have to take into consideration the effects on molecules. Although some usable DNA has been obtained from collections, the vast majority of collections, with current molecular techniques are not suitable for molecular work. Future collecting will have to collect for molecular work as well as morphological work. Except for the smallest of organisms it is possible to save a small part for molecular work and keep the rest of the specimen for morphological work.

The best method for killing specimens to obtain DNA is to place the specimen directly into liquid nitrogen and then freeze immediately at –80ºC (Dillon *et al*., 1996; Quicke *et al.*, 1999). However this then renders the specimen useless for morphological work so the identity of the specimen needs to be known beforehand. Also liquid nitrogen is difficult to obtain under field conditions. A compromise is to use ethanol of a concentration of between 70 and 100% here both morphological and molecular work can still be carried out (Reiss *et al*., 1995; Dillon *et al*., 1996; Quicke *et al.*, 1999). However ethanol is useless for butterflies and moths as the scales tend to fall off the wings when placed in ethanol making identifications impossible (*pers. ob.*). This can be overcome by removing the wings and placing them in a glassine envelope and just placing the body in ethanol (Brower, 1999). It is essential that all new material to be collected whether for DNA work or not should have method of killing labels and labels indicating any subsequent chemical or physical treatment attached to the specimen. This was not done for specimens currently held in collections and a researcher has no idea of the chemical or physical treatment history of a specimen.

3.1.4 Pest control measures and DNA

Although the killing, fixation and preservation of museum specimens pose many problems for the molecular researcher, pest control also can be just as damaging to molecules and is an essential part of the preservation of museum specimens. Chemical and physical treatments were routinely carried out for many years on specimens although many chemicals have now been withdraw for health and safety reasons. There are many pest control treatments available. It is worth noting that specimens preserved in alcohol do not suffer from pest attack. Dried specimens whether botanical or zoological however do suffer from pest attack. With many dried materials freezing at -30ºC for 72 hours or at –18ºC for 2 weeks is the current preferred method amongst museums. Heating to 60ºC is also a common pest control method although this can cause damage

to collections furniture (Ackery *et al., in press*). The Thermo Lignum® is another method that also uses heat but controls relative humidity and has a heating cooling cycle including three hours at 52ºC and this causes no damage to collections furniture (Ackery *et al., ibid.*). Botanical specimens are often subject to fumigation as a pest control method. Various fumigants are used such as methyl bromide, ethylene oxide, sulphuryl fluoride and carbon dioxide. There is very recent research into the effects of pest control measures and the effect on DNA. Kigawa *et al.* (2003) tested various methods on a freeze-dried fungi and chicken. They showed that all chemical treatments bar sulphuryl fluoride and carbon dioxide were detrimental to DNA. The sulphuryl fluoride treatment has also been deemed non-detrimental to DNA by Whitten *et al.* (1999). Physical treatment such as freezing and heating also appeared to not be detrimental to DNA (Kigawa *et al.,* 2003). The Thermo Lignum® method was also found not to affect DNA (Ackery *et al., in press*). However Kigawa *et al.* (2003) point out that they did not test for repeated pest control treatment of specimens and further work was needed to elucidate whether DNA would be affected by repeated pest control methods. Again I think it is essential that with any future pest control measures steps should be taken to keep information on what specimens were treated and what methods were used particularly with the chemical treatments that appear to render DNA unsuitable for study.

CHAPTER 4 - Storage of molecular collections

4.1 Storage of molecular collections

The storage of molecular collections presents new challenges for museums. Much is known about the storage and long term preservation of traditional natural history collections exemplified by the fact that many of the earliest specimens collected over 200 years ago are still in existence today. The fact that molecular collections need a completely different array of (expensive) equipment has far reaching effects on museum space and budgets. Molecular collections are generally kept deep frozen in freezers at -20ºC, -80ºC or in vats of liquid nitrogen at -155ºC. Most museums use freezers for storage of their molecular collections and only a very few museums have the resources for storage in liquid nitrogen.

4.1.1 The use of freezers for tissue and molecule storage

Freezers, whether -20ºC or-80ºC, are available in two models, either upright or chest. The advantage of the upright model is that it takes less floor space and gives better access to specimens whereas a chest freezer maintains a more constant temperature and is less prone to mechanical breakdown (Dessauer *et al*., 1996:37). When the freezer door is opened, particularly with -80ºC freezers rapid temperature rises will occur. This consumes a lot of energy, puts a strain on the mechanics of a freezer and could eventually contribute to freezer failure (Dessauer *et al*., *ibid.*). It is preferable that the contents of the freezer is databased or at least a map of the contents of the freezer is placed on the door thereby minimising time wasted while searching for samples within the freezer, causing problem outlined above. Frost-free freezers are not desirable as the temperature inside the freezer is raised temporarily to get rid of ice, and this can be extremely detrimental to the samples stored inside (Dessauer *et al*., *ibid.*).

4.1.2 Problems associated with freezers

The mechanical failures of freezers or power interruptions are a big threat to tissue collection security. Samples are easily lost if allowed to defrost. All freezers should have audible alarms in case of failure and these should not only be in the laboratory but also linked to the general museum security team. A list of staff telephone numbers, particularly during holiday period should be posted on the freezer and made available to general security staff. Some other form of backup such as another freezer, liquid nitrogen or dry ice should be available in case of failure and emergency generators also available in case of a power cut (Dessauer *et al*., 1996:37). The recent power cut across North-eastern America in August 2003 reinforces the need for back up generators. Luckily the major research collections were saved in this area due to back-up generators (Pearson, 2003).

4.1.3 Freezers and long term preservation of DNA

There also are serious doubts to the longevity of specimens stored in at -20ºC and at -80ºC. According to the American Museum of Natural History (2002) specimens held at -20º are subject to protein and lipid changes and damage from the growth of micro-organisms while specimens held at -80º are also subject to

protein and lipid changes, with extensive desiccation of specimens being observed upon light microscopic examination of frozen sections after only six months of storage, this degree of structural change may also induce some types of molecular change. This problem of long-term viability of samples is also echoed by Wood *et al.* (1999:269). This should be of great concern to natural history museums, as most that have molecular collections do not have the resources to store tissues in liquid nitrogen. Further research into the long-term viability of materials stored in freezers should be urgently carried out. If this is indeed the case the materials need to be stored below -130ºC then surely many museums are wasting valuable resources by not investing in liquid nitrogen storage.

4.1.4 The use of liquid nitrogen for tissue and molecule storage

The American Museum of Natural History (2002) advocate a colder is better regime and the majority of their collections at held in liquid nitrogen at -155ºC. Simione (1995:158) also state that animal and plant cells must be maintained below -130ºC to ensure long term stability. Liquid nitrogen is by far the best medium for long-term storage of tissues. It is worth noting that liquid nitrogen has only really been used for tissue storage in the last 30 years and only time will tell if it can keep tissues in a suitable state for molecular work indefinitely.

There are many problems with collections in liquid nitrogen. The first is the cost of setting up such a storage area, which can cost a minimum of £500,000 (see chapter 1.4.1 for details). This is a colossal amount of money for a museum to find from scratch and corporate sponsorship or collaboration with other institutes is likely to be the only way many museums can afford to set up and run a liquid nitrogen facility. Collecting of new material into liquid nitrogen in the field also presents problems. Few airlines are willing to transport material in liquid nitrogen and the availability of liquid nitrogen in developing countries is also problematic. The American Museum of Natural History does loan a field kit if the collection is to be deposited at the museum, included in the kit is a dry-shipper. The dry-shipper is a large flask, which can carry up to 80 small tubes. It contains liquid nitrogen in the vapour phase and it therefore allowed onto commercial aircraft. The contents may be kept at –155ºC for up to three weeks liquid nitrogen (American Museum of Natural History, 2003b). There are many health and safety issues concerning liquid nitrogen, which are outlined in chapter 4.2.

4.1.5 Databases for frozen tissue and molecular collections

The Freezerworks™ database is a tailor made database already in use by several museums throughout the world thereby allowing easy exchange of information. Many in the museum community have reservations about the ephemeral nature of tailor made databases. However Freezerworks™ is used right across the medical industry, worldwide, so there is little chance of the company behind it, Dataworks Developments, going into liquidation. The Freezerworks™ database tracks each bar-coded entry, has over eighty different data fields, including the specimen's placement in the collection, taxonomic identity, morphological voucher specimen catalogue number (or zoo animal identification number), tissue type and quantity, GenBank® accession numbers and bibliographic references associated with a given specimen (American Museum of Natural History, 2002). Freezerworks™ also has an easy to use web interface allowing collection of tissues and molecules to be made much more accessible to the researcher. One such example of a frozen tissue collection website using Freezerworks™ can be found the AM-CC at the American Museum of Natural History (American Museum of Natural History, 2003c).

4.2 Health and safety and tissue and molecular collections

There are many health and safety issues associated with molecular laboratories and in particular liquid nitrogen. Liquid nitrogen can cause severe burns and protective equipment such as goggles cryo-gloves, cryo-aprons must be worn at all time to avoid contact with liquid nitrogen (Simione, 1995:159). There is also a danger that when specimens are retrieved from liquid nitrogen that nitrogen may enter the atmosphere thereby lowering the oxygen content of the atmosphere. Oxygen monitoring alarms are essential in any liquid nitrogen facility. There are further health and safety issues concerning the reagents used in molecular biology, which are beyond the scope of this paper. Equipment such as centrifuges may also represent further hazards.

CHAPTER 5 - Museums and molecular work

5.1 Should museums be carrying out molecular work and be repositories for tissue collections?

As outlined earlier large museums have always conducted research into taxonomy and systematics and there is no reason why this should not continue just because there is a whole new method of answering these questions. There certainly is a perceived conflict between curators of natural history collections and molecular workers within the same institute. In the early days of molecular research whole specimens were destroyed in an attempt to locate usable DNA, often with no DNA being extracted (Austin *et al.* 1997). Most institutes now have policies in place regarding loan of material for molecular work and destructive sampling. In many cases specimens used for molecular work do not come from museum collections but are wild caught. However, museum specimens are used extensively to confirm identifications of the specimens concerned, which is often essential, as little will remain of the specimen after molecular work has taken place.

It is the case that museums should be repositories for molecular samples. Having had such a long history of maintaining collections of morphological specimens, it would seem only a natural progression that molecular samples should also be stored at museums. Museums often rely wholly or partly on public money for funding. Criteria for funding are often measured in output of scientific papers. A museum with a molecular laboratory is always going to publish more than a museum relying solely on morphological work. Systematics can now utilise a whole new selection of tools using molecular data and this can often compliment and confirm molecular studies. One complaint of traditional natural history curators is that molecular work is very expensive and diverts already tight budgets way from traditional collections, However molecular work often attracts large grants (unavailable for traditional natural history collections), which will include infrastructure costs, and this does benefit traditional natural history collections. Often molecular biologists have been accused of riding on the back of natural history museums. Many specimens collected by molecular biologists find their way into natural history collections, as sometimes only a small part of the specimens is required for molecular work. Clearly collaboration is needed between museum curators and molecular biologists working within the same institute. Collecting of new material can serve both a morphological and a molecular need. One question often asked is whether natural history museums are mimicking universities to survive. There should not be a conflict between natural history museums and universities. Many universities have natural history museums and many universities run courses associated with natural history museums (e.g. MSc in Taxonomy & Systematics jointly run with The Natural History Museum and Imperial College, London). This collaboration between museums and universities could solve a lot of the funding problems. Theodorides (*pers. comm.*) suggests that the cost of setting up liquid nitrogen facilities at The Natural History Museum, London could be shared with Imperial College, London.

5.1.1 Redundancy of molecular techniques

Molecular techniques evolve rapidly with new techniques being regularly developed and current techniques often become quickly redundant. There are six major techniques listed in Hillis *et al.* (1996:vii). According to Theodorides (*pers. comm.*) four of these are now obsolete. Not only are the techniques obsolete but so is a lot of the (expensive) equipment associated with these techniques. Any molecular laboratory will have to budget for the continuous replacement of equipment.

5.1 2 Do natural history museums need molecular biology to survive?

This is a bone of contention between traditional natural history curators and molecular workers. I believe that the future of systematics and taxonomy lies with molecular work. Certainly morphological work has its place but systematics and taxonomy relies more and more on molecular work to solve problems. I can envisage in 20 years time that systematics and taxonomy will be dependent on molecular work. Large natural history museums will have to embrace the molecular revolution otherwise the work of systematics and taxonomy will move elsewhere into the academic sector and funding bodies will fail to see the point of a large museum that does not use current techniques in its work. There is no doubt that molecular work gets far more funding than traditional morphological work. In order for museums to survive as leaders in systematics and taxonomy, the setting up of long term storage facilities for tissues and molecules with their associated laboratories is essential.

Collaboration in collecting of new material can greatly enhance large collections and the molecular worker should not be seen as the enemy by the traditional natural history curator.

CHAPTER 6 – Conclusions

6.1 Conclusions

There are many implications associated with frozen tissue and molecular collections and their impact on natural history collections. The most urgent of these is the cost of setting up and maintaining a frozen tissue and molecular collections. The cost of building these facilities runs into six figures and the maintenance is also a six-figure sum. At the moment few museums have a liquid nitrogen storage facility and the long-term preservation of tissues and molecules will require that they are stored in liquid nitrogen. Museum marketing people should be able to secure funds for a liquid nitrogen facility as many biotechnology companies have the resources available to sponsor such as facility. Running cost must be taken into account when such a facility is built. It would appear that if the large museums do not invest in liquid nitrogen facilities then a large amount of research into molecular systematics would just move to where such facilities are provided. If the research carried out did go elsewhere, this could well affect the funding museums rely on for their existence. Large natural history museums are going to have to invest in liquid nitrogen facilities to maintain their positions as world leaders in taxonomy and systematics.

The use of museum specimens for molecular work was fraught with problems in the earlier days, particularly regarding loan of material, destructive sampling and deposition of samples. At the present time most museums have policies in place regarding this issue. The issues of chemical and physical treatment of specimens during preservation and pest control measures have to some extent been addressed. However much more research is required into the effects of chemical and physical processes and a definitive list of safe and unsafe processes needs to be urgently drawn up. The collecting of new material for museum collections is an essential part of any large museum and consideration will have to be given to the methods used and their effect on molecules. Already many collections are made specifically for molecular or with molecular work in mind. It is also essential that specimens collected for molecular work also attempt to retain a voucher specimen for the morphological collection.

One can understand the concerns of curators and morphological workers of the indent molecular work is making on museums particularly where space and financial issues arise. Mistakes have been made in the past by both groups. Both morphological and molecular workers are working towards the same goal, that of better understanding of taxonomy and systematics of the natural world. There really should not be a conflict between the two groups and better communication is needed between both parties as they both have a lot to offer each other. Already there are curators of frozen tissue and molecular collections and more curators will be required as frozen tissue collections grow in size.

Systematics and taxonomy is relying more and more on molecular work to answer the many questions posed by the natural world. Although morphological work will not become redundant, molecular work will continue to increase in importance. Natural history museums have always carried out studies on systematics and taxonomy and to continue this work molecular biology must be embraced. Natural history museums have also acted as repositories for morphological specimens and it is logical that they must act as depositories for molecular specimens as well.

I believe that molecular collections are an important part of the future of natural history collections.

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