Editorial

Dear All,

I look forward to seeing those of you who can make the conference. And those that can't can catch the flavour of the days by looking over the published papers in the next Newsletter.

We are hoping to run an anoxic training workshop this year so please let us know if there are any specific issues that you would like to see discussed. Also, we have a list of potential training days on our website (www.nhm.ac.uk/hosted_sites/natSCA/training/seminars.html). Please let us know if there are topics that you would like to see covered that we are not considering. This is not a definitive list and we would value your input.

I'd like to welcome the new committee members on behalf of the whole committee - it's so important that we get a range of people from our members representing us on committee - it's the only way to stay up-to-date and fresh - and of course, to thank those who have left for all their hard work.

- Victoria Papworth

Contributions for Issue 12, July 2007

All articles, letters, news, adverts and other items for inclusion for the next issue of the NatSCA Newsletter should be sent to the address below by June 1st:

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Zinc Chloride in Liquid Preservation

- Kristian Murphy Gregersen

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Abstract:
For economical reasons, zinc chloride had been used as a substitute to alcohol for the preservation of anatomical and biological specimens in the 19th century. This lead to the degradation of many specimens, amongst other a great number of type specimens of sea snakes held at the Natural History Museum, London. The use of zinc chloride however seems to have changed around the time of the British Spirits Act in 1855, where after zinc chloride found a use as a specifically good fixative/preservative for brain tissue.

Introduction:
An investigation of the type specimen collection of sea snakes held at the Natural History Museum, London, in 2004, revealed that at least 75 % of the type specimens from before 1855 had suffered from decalcification due to preservation in zinc chloride (the details of the analysis will be presented in a paper at a later date). None of the specimens that were collected later than 1855 had any signs of decalcification, which lead to an investigation into the history of zinc chloride as a preservative. This paper presents the introduction and use of zinc chloride as a preservative, but will only deal with the 19th century, and will not concern the use of zinc chloride to-day.

Burnett's Solution:
Sir William Burnett (1779–1861), Director-General of the Naval Medical Department, was a Scottish surgeon who rose to the highest ranks within the British Royal Navy. He gained high admiration for his treatment of British prisoners of war, and his very effective methods in treating the sick and wounded. At periods he was stationed at the Royal Naval Hospital, Haslar, in Portsmouth. Here he opened a library and a museum, and built up a considerable collection of natural history specimens, housed at the Haslar Museum until its transfer to the Natural History Museum, London, in 1855. (Anonymous 1833, Anonymous 1850, Anonymous 1861)
On the 26th of July 1839 Burnett received the approval of his patent application from 1838, entitled "*Improvements in preserving wood and other vegetable matters from decay*" (Burnett 1838). His invention makes the use of zinc chloride (1 pound of zinc chloride to 5 gallons of water) to preserve wood, canvas, cordage, sailcloth, hemp, flax etc., simply by soaking the object in the solution (Burnett 1838), thus hardening and improving the texture of the material greatly (Anonymous 1847). Later it was observed to be efficient as an antiseptic remedy in the treatment of wounds, ulcers, and bad odour, and became known as *Burnett's Solution* (Wallace 1848, Anonymous 1861, de Morgan 1866, Crace-Calvert 1872a&b, Anonymous 1905) with the specific gravity of 1.343 (Rolleston 1879).

A preserving agent for biological specimens:
At some point, soon after the application of zinc chloride as an antiseptic, it seems to have caught on as a liquid for the preservation of anatomical and zoological specimens ("A Student" 1848, Bryson 1848, Anonymous 1872, Anonymous 1877). It was cheaper than alcohol, which was heavily taxed, and easy to carry around in the form of a powder soluble in water (Nassau 1892). It was clearly used at the Haslar Museum (Günther 1912) due to the influence by Burnett, but otherwise there are no direct suggestions that show other institutions used it. In fact, the only citations I have been able to find on the use of zinc chloride as a preservative are all concerned with the preservation of whole human bodies for dissection. It could be due to the fact that very little was published about conservation in those days, and that my main source, *The Lancet*, is a medical journal. It might also be due to observations of its bad effect on the specimens preserved in the liquid, evident from a footnote on page 5 of Günther's (1912) "*General History of the Department of Zoology*":

> "‡ *The zoological collection at the Haslar Hospital (…) were in very bad condition, as for economy's sake a solution of chloride of zinc had been used instead of alcohol!*"

Even so, most of the type specimens of sea snakes at the Natural History Museum, London, had been preserved in this way, suggesting they must have used the same liquid.

Though it never really seems to have caught on as a general preservative, it seems to have been preferred as a fixative/preservative for brains and other nerve tissue. Chapman (1880) used it to preserve brains of gorillas, and John B. Roberts (1885), M.D., used brains from whole human cadavers preserved in zinc chloride. Professor von Bischoff of Munich had in 1868 used it to preserve brains for 24 years and found it far superior to alcohol, and the Oxford Museum also used it as a brain preservative (Rolleston 1879). Zinc chloride resulted in much shrinkage of the tissue though, and some found it better to use it as a fixative, before transferring the brain to a different solution for preservation. Professor Turner (1879) used this method and would fix the brain in a saturated solution of zinc chloride before transferring to alcohol, and later glycerine and carbolic acid solution, before finally varnishing it with gum elastic or marine glue. Parker and Floyd (1895) also noted the shrinkage effect of zinc chloride and tried to find a mixture that would prevent this. Formaldehyde had recently immerged as a fixative, but had the effect of swelling the nervous tissue. Therefore Parker and Floyd tested them together but found that the results were not satisfactory, and advised against the use of zinc chloride. Pierre A. Fish (1896) followed up on the same idea as Parker and Floyd and but thought of adding sodium chloride to make the following fixative:

Water,	2000 cc.
Formalin,	50 cc.
Sodium chloride,	100 grams
Zinc chloride,	15 grams

The mixture should have the specific gravity of 1.05 and the brain should be fixed in the solution for c. 10 days, before transferring to formaldehyde or alcohol for storage.

Discussion:
Burnett found the usefulness in zinc chloride as a preservative and, probably through his position in the Royal Navy (Anonymous 1861), boosted the use of it within the navy. There was a very high tax on alcohol in those days which also induced the replacement of such with a far cheaper remedy. It is probably safe to venture that all of the specimens held at Royal Hospital Haslar were preserved in zinc chloride, and if one takes a look at the collectors of the type specimens of sea snakes held at the Natural History Museum, London, most of them were collected by people like Major-General Thomas Hardwicke (1755-1835), Admiral of the British Royal Navy Sir Edward Belcher (1799-1877), and Surgeon Major Thomas C. Jerdon (1811-1872), all connected with the British Military and therefore under heavy influence from Burnett. The only odd-one-out is Major-General Hardwicke, who died before Burnett got his patent in 1839, and before zinc chloride was found to have antiseptic properties. The only explanation for this is that the original preservative for those specimens must have been replaced by zinc chloride at a later time. A very thorough book on the preservation of anatomical specimens by Usher Parsons (1831) suggests that zinc chloride was not a known preservative, or even used as a salt added to preservatives, before Burnett got his patent.
In 1855 the *British Spirits Act* was passed, removing the tax from alcohol for industrial use after the addition of methanol which made it un-suitable for consumption. This type of alcohol is referred to as Industrial Methylated Spirit (IMS) and quickly caught on as the substitution for the more expensive alcohol and is still utilised by the Natural History Museum, London. This co-elates with the results of the investigation of the type collection of sea snakes, which found that the signs of decalcification due to the action of zinc chloride were not to be found in specimens later than 1855, suggesting that shortly after the tax was lifted on industrial alcohol, zinc chloride went out of fashion, except as a brain fixative/preservative. This was likely due to the observations of the ill effects it had on the specimens, like Günther (1912) mentions.
Zinc chloride enjoyed but a short period as a general preservative (c. 30 yrs.), and only, it seems, within the British realm.

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Giving The People What They Want
Meeting Zoology Collections With Their Audiences: A Case Study

- **Jack Ashby, Grant Museum of Zoology**

In 2005 the Grant Museum of Zoology at UCL took part in a London Museums Hub project *Say it Again, Say it Differently*, aiming to completely reinterpret the Museum. The Grant Museum (GMZ) was founded in 1827 as a teaching resource for UCL students, and despite opening its doors to the public ten years ago, had not done enough to make the collection into a useable space for non-academics. Below I outline what the GMZ did to generate accessible interpretation to encourage new audiences into the Museum for the first time in 180 years, and why. I discuss the understanding and appreciation of natural history by the public and how to make use of it.

Background

The GMZ is a fairly typical Victorian natural history collection: tens of thousands of skulls, skins, skeletons and wet specimens displayed extremely densely in wooden cases. It was founded by Robert Grant, a radical evolutionist and the country's first Professor of Zoology and Comparative Anatomy, at the University of London. Grant is famous for his influence on the young Darwin in Edinburgh, and is considered to be the first person to teach evolutionary theory at an English university, using our collection to do it. The collection is displayed taxonomically, as it was in Grant's day. There are a number of hero objects including one of only seven quagga skeletons, dodo bones and a pickled thylacine dissected by Thomas Henry Huxley.

In 1995 the Museum opened its doors to the public, and since then the small number of staff have been working to welcome new audiences into the Museum. A Learning and Access Programme was established in 2004 to develop services for the Museum's audiences, and with it the opportunity to create new interpretation for all visitors. The process we undertook in our re-interpretation can be rolled out into other natural history galleries and museums and need not be exclusive to exhibitions: it suits any audience-geared project.

1) Choosing an audience – who are you talking to?

The first task in any engagement exercise is to select an audience. It helps to be as specific as possible. In writing an exhibition the tone of voice, design, size, number and indeed the height of the labels from the floor will vary between age-groups. This decision must be made first as it directs all other work. The choice may be obvious in that you are building a new set of interactives for a family gallery, but in the case of the GMZ we were redisplaying the whole museum and had to consider all of our user groups.

People can use the Grant Museum in a number of ways: as general visitors during public opening hours (weekday afternoons), as part of the schools programme, as part of University teaching, or on an educational activity day. Of these audiences, specimen-based facilitated workshops and activity days cater for families and schools and UCL teaching provides for the students; it was the general visitors who were not being provided for. As a result of the opening hours, most of these visitors are adults. We decided that the new interpretation would be for them.

2) Establishing goals – why are you talking to them?

With any audience-based project, clear outcomes for the museum should be established at the outset. Why is a project going ahead? What does the museum want the users to go away with at the end? Two types of measure are set: simple statistical goals (How many visitors? How long did they stay? How much merchandise did they buy? How many repeat visits? etc), and learning outcomes. It is sensible to be general when setting educational targets – specific pieces of information may be included, but be realistic about how many pieces of information visitors will experience on a visit. Most people will not read every label in a gallery – just listing content to be memorised from your interpretation does not constitute useful learning outcomes. For our project, we developed a short set of general learning outcomes based on the Inspiring Learning for All framework (MLA, 2004), which would be tested before and after the work. These were:

To learn facts about the collection.
To increase appreciation for the natural world.
To be surprised and inspired by the museum displays and to enjoy what they see and do.

As important as what a museum wants to do in a project, is what the target audience wants to get out of it. We needed to establish what our non-specialist adult audience wanted from the Museum? The staff had their own ideas of the Museum's short-comings: the existing labels were tiny and handwritten in Latin; visitors didn't understand the taxonomy and no information was being provided about the animals represented.

3) Selecting themes – what are you going to say?

Natural history is an unusual science; perhaps more than in any other field, the layman can be extremely well informed. Unlike the other sciences, and particularly the more physical disciplines, people are very regularly exposed to intelligible, undisguised and undiluted information about the natural world. Natural history documentaries are an obvious method of public science communication, and many people can even source almost all of their learned knowledge to a single man on the BBC. However the topic is far more prevalent than even that: the physical adaptations of the platypus have been used to advertise crisps; animal species frequent linguistic idioms and sayings; thousands of creatures are used as accurately interpreted fictional characters; and Darwinian metaphors are rife in the world of sport. When engaging with museum audiences, it is crucial to realise that people will often know what you are talking about.

We knew what we wanted to say to our audience. The specimens have a great deal of history themselves, as well as the natural history of the species they represent. Five main themes were identified: specimen history, natural history, ecological conservation, scientific research and mythology. Once themes have been established it is important to be strict when developing content. If it doesn't fit into the overall stories you are trying to tell, it ought to be left out. Being consistent will help visitors go away with the topics you want discussed.

4) Evaluation – what do they want to know?

Knowing what you're doing wrong and what you want to do about it is all very well, but it is very dangerous to imagine what an audience think and want without actually asking them: evaluation is critical.

The GMZ set up a focus group representing adults of varying age and background, and they met with a team of consultants before work began. We felt it was important that external consultants led discussions so that no biases were introduced. This involves more costs than running sessions yourselves, but the data are more reliable this way.

Evaluation can be a three stage process (Hooper-Greenhill, 1994): baseline (to identify goals before a project), formative (to ensure progress is occurring in the right way during a project) and summative (to make minor changes after the product is launched). Formative evaluation can take place as many times as needed, and is arguably the most valuable part of the process.

Thankfully our focus group agreed with what we wanted to say at the baseline stage, and also agreed with our ideas about why the Museum was not delivering: "It feels very cramped, it's like you've wandered into a mad collector's living room – all jumbled together". Many people felt confused "all crammed together, I don't know what's what or what information goes with what". In addition they brought new ideas we hadn't considered.

5) Meeting the audiences' goals – how do you use evaluation?

Evaluation as a developmental exercise is pointless if it happens only after a project has finished or if you don't take into account the outcomes. There are examples of museums replacing focus groups like a monarch sacking parliament when they don't like what they hear. Problems can arise when a museum's ideas conflict with focus groups' and they are hard to resolve. Arguments that an evaluation team is too small to be a representative sample of the audience are not necessarily invalid, but it would take a very good reason, and a lot of bravery, to ignore well-researched evaluation. To avoid uncertainty, think hard about what a focus group is being asked and word your questions well. Do not put words in their mouths and don't cherry pick the bits that you agree with. Satisfying their suggestions requires effort, but if you don't intend to listen to them don't ask. Developing a project which deliberately ignores an audience's view will alienate them to a greater extent than one that doesn't ask their opinion in the first place.

As with many museums that are trying to open themselves up to new visitors, the GMZ was concerned that the changes that they needed to make might upset or alienate our original audience, in our case the UCL community of staff and students. It was considered crucial that this existing audience was as in-

volved in the developments as the new one, and so a second set of focus groups was established for them and asked the same questions.

Education and exhibitions staff across the sector have long-lamented their conflicts with curatorial and academic staff. It is important that the two work together and both meet their own strategic aims. However, it must always be kept in mind that while curators and academics are often the experts in their field, they may not be the best people to interpret their subject to the public. Understanding what an audience knows and what they will be able to learn are often the points at which interpretation can succeed or fail. With zoological objects, while people do know a lot about animals, they do not know a lot about skeletons or body parts (Tunnicliffe, 1998).

Some of the academics in our UCL focus group were keen that visitors were encouraged to work out what animal a skeleton came from themselves, while education staff said it would take a non-specialist too much thinking to interpret several hundred specimens in one visit. Discussions took place and we managed to convince them that skeletons should be accompanied by the image of the living species to aid interpretation (Tunnicliffe, 1998). Debate can only happen if you lead the focus groups yourselves, and we felt that it was appropriate for us to run the internal UCL focus group as our own colleagues were likely to be more candid and critical to our faces than the public group.

Discussion led they way through the focus groups and in the end we came out with an interpretation strategy to develop the Museum as a new public-space. The look and feel of the Victorian collection wouldn't be changed, but the labelling would transform the space into a valuable learning environment.

At the end of the first stage of evaluation the focus groups were given prototypes of proposed labels to comment on, starting the formative phase early. The feedback was very positive and the comments extremely constructive. It was thoroughly worthwhile to get approval from the audiences at a very early stage. With this in hand, huge amounts of progress can be made with the confidence that developments will be successful.

6) Develop new ideas – unite what you want to say with what they want to hear

One of the outcomes of the focus groups was that they wanted to know what every specimen was. How we were to do this presented a challenge: some cases house over one thousand objects – one thousand labels would fit in as well. What information would be useful? It would certainly not be possible or practical to give everything a name. There may be five hundred species of gastropod in a display, but the public would not want to know all of their names.

Taxonomy was the key. Most members of the public would not feel able to define taxonomy, but I would claim that to some extent they know it when they see it; the principles at least. People instinctively put things into groups, whether they are zoologically accurate or not. The interpretation of natural history for a public audience can tap into this. What the Grant Museum did is to affix a tiny label depicting the outline of an animal to most specimens. Taxonomic groups were assigned a representative animal and each member of that group was then united by a common icon. Each bony fish had a perch icon on it, whether it was an eel or a salmon, and each insect, from beetle to phasmid was represented by a wasp.

This icon-based taxonomy was the lowest rung of a four-tiered hierarchy of information. If nothing else, it labelled every specimen as a member of a group. It was a gastropod, a bat, a marsupial, a crustacean etc. It also challenged people's preconceived groupings, whales as mammals for example, and underlined the relatedness of living things. Specimens were then selected to be individually named, labelled with brief interpretation, and large labels represented an entire case. This interpretive strategy was built through formative evaluation by the audience focus groups.

Exhibition label writing is a constant battle between what there is to say, what the audience want to discover, how to say it and keeping text to a minimum. A case of specimens can be visually ruined by the presence of too many labels, or too much text. The stories the Grant Museum's specimens had to tell, and the information the audience wanted to know would not fit in the cases. Our solution was to provide the interpretation outside the cabinet – in the form of audio-guides and hand-held Factfiles. The specimens represented in this way are numbered so that if the visitor wants more information they can get it from the hand-held tools.

Throughout the whole project, which took over a year, progress was determined by the comments of the focus groups and the extent to which the learning outcomes were met. No redisplay or reinterpretation should happen without consultation with the intended audience, and curators cannot be given free reign. Sacrifices may well need to be made with regards to the information conveyed.

A year has passed since the new interpretation was launched. The Grant Museum can report a 600% increase in our annual users since 2004, and dramatic (though unmeasured) improvements in visitor dwell-time. This is thanks in part to well-designed evaluation by our consultants, as well as the enhanced programme of events in out Learning and Access Programme.

Conclusion

Natural history collections are in a very lucky position: they do not need to start from scratch. Thanks to the media people know a lot about animals, plants and the environment, and often they know a great deal of detail about specific organisms. While visitors to museums may not be able to identify a skeleton of a certain animal, they may well be able to take a lot away from seeing it without being told anything but the name. Once visitors have been pointed to a bat skeleton, for example, they do not need to be told how they fly because they can see the bones of the hands for themselves. There is a balance to be made between highlighting information that is already known in this way and telling the specimens' stories that will be new to visitors. This balance can only be struck with a detailed understanding of what people already know and what they are interested in once they are told for the first time. To find out what this is you have to ask them.

Pandering to modern popular culture by highlighting animals that are fresh in an audience's psyche due to inclusion in a recent Disney film may leave a bad taste in the mouth, but it will relate to your visitor. Natural history is everywhere, through dialogue with evaluators museum staff can gauge what is known and what people want to hear.

If you would like a free copy of the best practice guide produced by the London Museums Hub as a result of the *Say it Again, Say it Differently* project then please contact me at j.ashby@ucl.ac.uk (limited numbers available). Other comments and questions are welcome.

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In search of an Emperor Penguin

The Natural History Museum in London are looking for a smart looking display specimen of an Emperor Penguin to exhibit in our forthcoming blockbuster exhibition, *Ice Station Antarctica*. The exhibition will be touring internationally for 5 years from 2008. We are therefore looking for a taxidermy specimen with minimal conservation requirements (and therefore provenance) that can form part of a travelling exhibition.

Do you have an Emperor Penguin that might be suitable? If so, please contact Emma Freeman at the Natural History Museum (contact details below).

The Natural History Museum are producing *Ice Station Antarctica* in partnership with the British Antarctic survey. It opens at the Natural History Museum from 25th May 2007.

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Nature Detectives
- Kate Measures, Nature Detectives Trainer, Heritage Learning Consultant

21st century environmental science opportunity hits museums
This winter, heritage staff and volunteers in the north of England have been attending free Nature Detective training. The Woodland Trust's Nature Detectives Project is an online environmental science project enabling children to develop a lifelong love of nature and concern for our natural heritage. The project links perfectly with many of our heritage sites and museum collections.

What's it all about?
This HLF funded project is the junior wing of the UK Phenology Network – a partnership between The Woodland Trust and the Centre for Ecology and Hydrology. It is closely also connected to BBC Spring and Autumn Watch programming. Both are based around the science of phenology – the study of the seasons and seasonal changes. Nature Detectives explore the timing of natural seasonal events such as bud burst, migrations and fruiting of common species. The data collected is used , amongst other things, to help track the effects of climate change on our flora and fauna.

What's the history?
Numerous founders of natural history collections and indeed museums themselves were Victorian or Edwardian naturalists and philanthropists. The very same people were recording phenological data. Phenological data held by the Centre for Ecology and Hydrology spans back to 1736. This was when the forefather of modern phenology, Robert Marsham began recording 'Indications of Spring' on his family estate near Norwich, Norfolk. He continued to note down significant dates for the next 62 years. His main reason for keeping these records was to improve timber production on his estate but this provides a valuable source of comparative data for us today.

There is no doubt that other of our large estates or some of the founders of our collections and museums also kept such records, (perhaps not so systematically) over the last 200 or so years for game keeping, forestry, groundsmanship or just general interest.

The Royal Metrological Society coordinated an official recording scheme between 1875 and 1947. This was done nationwide and is the source of some of the richest historical data. The records were kept to examine the relationship between meteorological events and the natural world. But the UK Phenology Network didn't start recording until 1998 so where does the data in the middle come from?
Records can turn up in personal diaries, records of estates, museums or archives but also pop up in the most surprising of places such as the door of this garden shed painted with flowering dates of daffodils!

How can museums benefit from the Nature Detectives project?
The project is supported by a fabulous colourful and lively website powered by an excellent database of records. Museums, schools and any other group can access the website at www.naturedetectives.org.uk and download resources, activities and factsheets for free. These can be used as part of existing school and family natural science activity programmes.

Better still, museums can offer Nature Detectives sessions for groups and start recording phonological data at your site. Thousands of schools are already involved in the project and are actively recording data at their schools. Natural science collections can support identification skills and researching historical data. Our museums can provide safe and inspiring venues for learning identification skills, researching historical data and outdoor spaces for recording. Recording as part of Nature Detectives could also provide the prospect of promoting local biological recording schemes and an accessible way for children and schools to get involved.

This is an excellent opportunity to link our natural science collections directly with up-to-the-minute climate change science and for our visitors to input valuable data to the project.

For more information about the project look at the Nature detectives website: www.naturedetectives.org.uk

A preliminary investigation into using Tyvek® bags for short-term storage as a means of protecting herbaria from damage by insect pests such as *Stegobium paniceum*

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Abstract
A preliminary investigation into the properties of Tyvek® as a physical barrier against insect pests, especially for use with botanical specimens, was undertaken. The physical properties of Tyvek® were investigated using SEM to establish the structure and its suitability as a barrier against insect pests. Various designs of Tyvek® enclosures were produced using heat sealers and plastic sealing clips for standard-size herbarium sheets. The conclusion is that this may be an effective tool to support museum-wide IPM regulations and will be subject to further testing.

Introduction
The aim of this project is to investigate Tyvek® as an option for temporary storage of herbaria to protect them from attack by insect pests while in transit as part of a museum-wide Integrated Pest Management (IPM) strategy, or when taken out of normal storage for a period of time.

Herbaria
These were traditionally bound in book form (Fig. 1) but which are now more commonly stored in folders (Fig. 2). These folders are kept in either wooden or metal cabinets. A herbarium consists of a collection of dried and pressed leaves, flowers and fruit together with information on habit, morphology, anatomy, biochemistry, ecology and distribution of the specimen in question (Clark, 1988; Gunn, 1994). This information can be handwritten in ink or pencil, or are printed. The specimens are mounted on conservation grade 'rag' paper and nowadays with reversible polyvinyl acetate adhesive. Historically, wheat starch paste, animal glue (including fish glue) and methyl cellulose were used to attach specimens, and even being left loose within folders has been an option, leaving them vulnerable to insect attach which if left unchecked can be catastrophic. Together with the plants, the storage cabinets, paper and glues can all provide food and/or shelter for insect pests.

Fig. 1. Herbaria in good condition (Courtesy of The Natural History Museum)

The botanical collection at the NHM includes over five million specimens of myxomycetes, lichens, algae, bryophytes, pteridophytes and seed plants, of which 120,000 are type specimens. All the geographical regions are represented. Additionally, many of the collections are of historic importance, as for example those of Sloane, Hermann, Banks and Solander. In common with other herbaria the collection is actively used by researchers and visitors both to and from outside the Museum via loan agreements. For example, during 1991 and 1992 it was estimated that 14,000 specimens were sent out on loan (Huxley, 1994). The demand on the collection is, therefore, very high.

Fig. 2. Herbarium sheets stored in a folder

Insect pests of herbaria
Well documented examples of damage caused by insects on herbarium specimens exist; these include the biscuit/drug store beetle *Stegobium paniceum* (Fig. 3) and the cigarette/biscuit beetle *Lasioderma servicorna* (Bridson & Forman, 1998) which feed directly on the preserved plant material. They will eat all dried stored products, and even substances that to other insects are toxic – they have even been found feeding on strychnine (Hickin, 1985; Florian, 1997; Bridson & Forman, 1998), as well as papier maché,

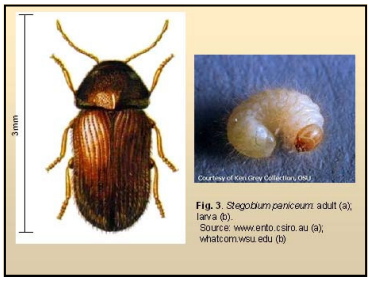

Fig. 3. *Stegobium paniceum*: adult (a); larva (b).
Source: www.ento.csiro.au (a); whatcom.wsu.edu (b)

freeze-dried animal specimens and fungi. They have also been recorded feeding on chocolate and on the leather spines of books (Hickin, 1985; Pinniger, 2001). They can tunnel through hard materials, and when first hatched only measure 0.5 x 0.125 mm and so can easily penetrate packaged foods. The development time depends on their supply of food and temperature, e.g. with a good food source and at 30 °C they can develop within five weeks. At lower temperatures the life cycle can be anything from six months to one year (Pinniger, 2001). Large numbers can be supported by a relatively small food source. Hundreds of beetles were found in a museum store where they had been breeding in a few old loose grain mouse baits (Pinniger, 2001). When the adults emerge they make neat round exit holes (Fig.4) and live for six to eight weeks. The damage from larvae can be quite extensive and even total destruction of plant specimens (Figs. 5, 6, 7).

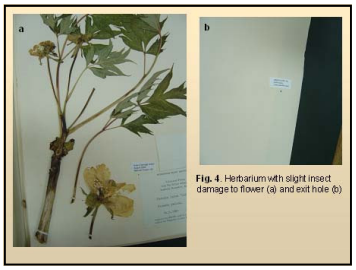

Fig. 4. Herbarium with slight insect damage to flower (a) and exit hole (b)

Fig. 5. Extensive insect damage to flower

Fig. 6. Verso of Fig. 5 showing extensive insect damage to paper support

Fig. 7. Complete destruction of plant specimen by an insect

Other insect pests of herbaria are those that feed on mould and detritus. In small numbers they can be a nuisance but large populations can cause serious damage. These include: spider beetles (e.g. golden spider beetle *Niptus hololeucus*), *Cartodere filum* (a small, slender beetle measuring 1.2–1.6 mm), silver fish (*Lepisma saccharina*), booklice (*Liposcelis* spp.), cockroaches, ants, carpet beetles and moths.

Pests are also resourceful in their ability to survive, e.g. *Tineola bisselliella* the webbing cloths moth has been recorded eating its way out of plastic bags in which bone specimens were stored (Kerr, 2005).

Control of insects and IPM

Historical control of insect infestations was largely based on chemical treatments such as methyl bromide or dichlorvos (marketed as Vapona), but most of these chemicals have now been withdrawn from use because of health and safety implications. Furthermore, the mounted herbarium sheets were historically treated with mercuric chloride, inorganic arsenic trioxide, barium fluorosilicate or organic pesticides including lindane, DDT and naphthalene. The residues of these are long lasting and extremely detrimental to health (Clark, 1988; Purewal, 2001). Freezing is now a preferred method, but other methods of insect pest control are being investigated (Åkerlund & Bergh, 2001; Warren, 2001; Conyers, 2001; Ackery *et al.,* 2005; Beiner & Ogilvie, 2006). These methods, however, are to treat infestations and as such do not give permanent protection, therefore good housekeeping practice is paramount, a point strongly emphasised by Child (1994), Blyth (2001) and Kinglsey & Pinniger, 2001).

To deal with issues of pest control a holistic approach known as Integrated Pest Management (IPM) are being introduced in many institutions. It involves institution-wide monitoring for pests, targeting treatment only where it is needed and modifying the environment to discourage pest attack (Pinniger, 2001).

Specifically, good housekeeping practices for keeping reinfestation to a minimum is essential as part of the IPM strategy. The NHM has implemented a system of identifying Risk Zones throughout the museum which incorporates IPM procedures such as trapping and regular monitoring.

Tyvek®

To help comply with IPM regulations, Tyvek® bags could prove to be a valuable contribution.

A search of the published literature showed that few studies had been done examining Tyvek® as a pre-

ventive conservation material. Only one independent study (Walker, 1986) was found that had looked in detail at the properties of Tyvek® as a storage material for artefacts. Generally, statements were made in the literature merely that Tyvek® had been used, but gave no further details. Additionally, research done by DuPont tends to published in its own journal (mainly in the US issue, which is not available in the UK), although some of it is also published on their Tyvek® website. It appears, therefore, that most of the information available on its effectiveness as a conservation material is anecdotal.

Tyvek® is the registered trade name for DuPont's spunbonded olefin, which is made from very fine, 0.5–10 µm high density polyethylene fibres (for comparison, human hair is *c*. 75 µm in cross-section). The fibres are spun, laid randomly and then bonded together by heat and pressure without using any adhesives or fillers (Anon., 1986; DuPont, 2002). The result is a bright white 'strong, lightweight, flexible, smooth, low-linting, opaque and resistant to water, chemicals, abrasion and aging' material that is also resistant to punctures and tearing, it is pH neutral (pH 7) and it is breathable (DuPont, 2002). Coloured varieties of Tyvek® are now also available, although white is preferred by conservators due to colour fastness concerns.

Three types of Tyvek® are available – types 10, 14 and 16. Type 10 is a smooth, stiff non-directional material resembling paper whereas types 14 and 16 are 'soft' and fabric-like. They are made in the same way but the finishing process determines whether it is the 'hard' type or the 'soft' type.

Tyvek® is widely used in medical sterile packaging because it is bacteria resistant, as a membrane and air filtration barrier in house building, for maps, charts and other printed material, wrapping materials and as envelopes. Its original use in conservation was for labelling archaeological objects from excavation sites (Winsor & Ball, n.d.) but has also been used for spine repair on books, as a book covering material, as dust sheets, as protective clothing and for labelling specimens. Tyvek® can be corona treated, which increases its ability to take ink (DuPont, 2002), have an anti-static treatment (potassium dibutylphosphate), but which is damaging to metals (Preserv'Art, 2005) or both surface treatments. If, however, the Tyvek® is to be used for sterile medical packaging, food or toys the surface is left untreated. Commercially, Tyvek® is recommended for: use in exhibitions; interleaving; envelopes, folders, portfolios; shipping cases; supports; packing; protection against dust and/or abrasion; documentation; and for storage.

DuPont considered that Tyvek® would not be a suitable material to use as a barrier against insects but acknowledged that they had not done any work in this area as it was not of immediate concern to them (pers. comm., 2006).

Methods

To assess whether there was a need for something like a Tyvek® bag to prevent insect attack of herbaria, a query was posted on a conservation website and informal discussions were had with a number of librarians and others interested in insect pest management (e.g. at the PRE-MAL Symposium, Stockholm, 2005).

Suppliers and the manufacturer were contacted in order to establish which Tyvek®-type was in use museum-wide.

Scanning electron microscope (SEM) images, using a variable pressure scanning electron microscope (Leo 1455VP) at the NHM, were taken at various magnifications (ranging from 80X to 500X) to identify the structure.

To create the bags a Crossweld® heat sealer (impulse sealer) 240 volt Mark 4 with a self-adhesive silicone coated barrier tape covering the jaws (Fig. 8) was used at various settings until a setting that was reliable and reproducible for heat sealing Tyvek® was found. This was also confirmed by SEM analysis. Once this was established, test bags were made large enough to hold standard herbarium sheets measuring 445 x 285 mm.

Fig. 8. Crossweld heat sealer (reproduced with permission from the Natural History Museum)

As the bags were made from one sheet of Tyvek® folded and heat sealed on two sides only, types of secure closure were sourced. Escal® strips currently used in the Palaeontology Department for anoxic environments and WeLoc® clips (marketed as Klippits™ for food bags),

originally designed for use within hospitals to close bags of fluids were tested (Fig. 9).

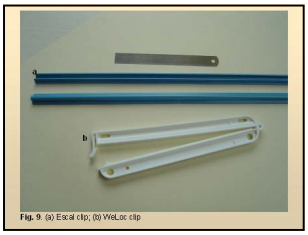
Fig. 9. (a) Escal clip; (b) VeLoc clip

Samples of *Spirea* sp., *Mahonia* sp., sweet flowering cherry and flowering red current, which are attractive to insects, were obtained from a local garden centre[2]. These were prepared by placing them between blotting paper layered between newspaper and pressed under a heavy weight for three weeks to dry and then standard mounted. Once they were dried the plants were mounted by the Botany Department in the style used at the NHM. These are now waiting to be used in the insect trials as Phase 2 of the project.

Discussion

To comply with IPM regulations, it is generally agreed that a short-term barrier against insects for museum objects is required. For botanical collections this would include when herbaria are taken out of normal storage for study and left exposed in a working environment, or when being moved around the building to other departments where IPM regulations are less restrictive (e.g. when waiting to be photographed: A. Paul, pers. comm., 2005). Also herbaria are sent outside the Museum on loan, these may be through the standard postal system or couriered depending on the nature or condition of the herbarium in question. Some form of bagging system would also be useful for when new material arrives in the department which cannot be processed immediately with quarantine procedures (e.g. freezing).

It was established that three types of Tyvek® existed – types 10, 14 and 16. Type 10 is easy to identify as it is a stiff material that looks very much like paper and is marketed as such. Most people will be familiar with this type as it is used for the near indestructible white envelopes. Types 14 and 16, referred to as 'soft', are fabric-like, but from the literature it could not establish which type was used at the Museum. Neither Conservation by Design nor Conservation Resources Ltd, who both supply the material-like Tyvek®, were able to help me on this point. Eventually it was discovered that standard UK supplies were of type 14 with a weight of 43 gsm (1443R). A heavier weight (73 gsm) Tyvek® type 14 (1473R) also exists but this is not available in the UK.

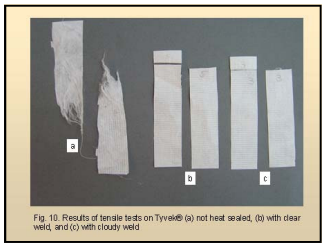
Fig. 10. Results of tensile tests on Tyvek® (a) not heat sealed, (b) with clear weld, and (c) with cloudy weld

The settings on the Crossweld® heat sealer that worked with consistent results were: weld 3 and 6 cool. However, on closer inspection it found that within the clear area of the sealed welds were small cloudy portions along its length. To see whether these cloudy areas affected the seal it was decided to do a tensile test (using Instron 4411). Although Tyvek® is not affected by such environmental conditions, the test was done at room temperature (23 ^{o}C) with an RH of 50%. Strips measuring 95 x 20 mm, 10 mm to weld were cut from the clear areas and from the cloudy areas. As a control, non-heat sealed Tyvek® was tested which stretched and distorted and then tore away producing ragged fibres (Fig. 10). It did not snap apart. Both the clear weld only strips and the strips with cloudy and clear welds easily tore, although the strips with only the clear areas snapped suddenly whereas the strips with the cloudy areas opened up along the edge of the weld and peeled away suggesting areas of weakness. To make them more secure it was decided that the Tyvek® bags would need to be double sealed. By double sealing it was hoped that the cloudy areas would be offset from each other thus reducing the areas of weakness.

On further investigation of DuPont's literature it was found that the antistatic and the corona surface treatments can affect the uniformity of the heat seal. To overcome this it is suggested that a low density polyethylene coating is used with the Tyvek® when heat sealing it.

In the Palaeontology Department, Tyvek® cushions filled with Plastazote® 'chips' are used to protect delicate specimens when being examined. These are made using the Crossweld® heat sealer, but these seals do not have to be perfect as they are not being handled to any great extent and are only a temporary measure.

Further analysis by SEM taken at various magnifications confirmed the non-directional structure of the fibres that make up Tyvek® (Figs. 11 & 12). We intend to do further study to look at the 3-D structure to see how likely it is that a *Stegobium paniceum* larvae could push its way through the fibres, or for the

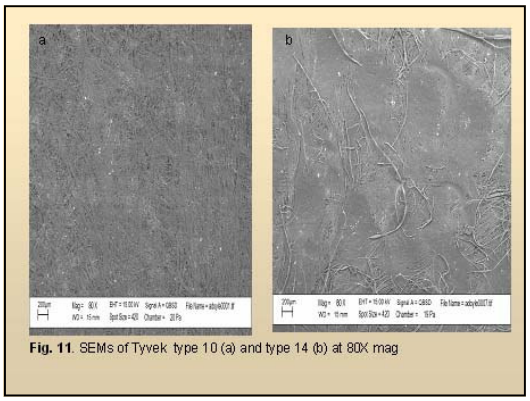

Fig. 11. SEMs of Tyvek type 10 (a) and type 14 (b) at 80X mag

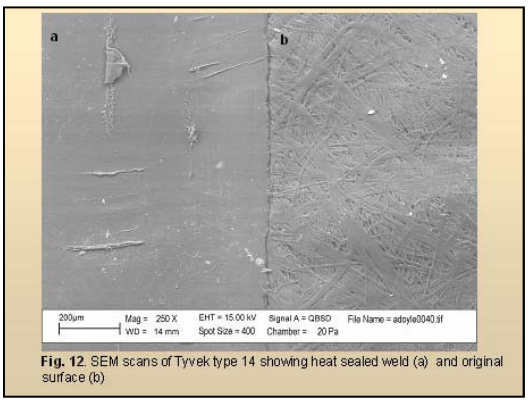

Fig. 12. SEM scans of Tyvek type 14 showing heat sealed weld (a) and original surface (b)

adults and larvae to chew their way through it. The medical packaging section of DuPont's website states that the pore size of Tyvek® is 0.5 µm and with the non-directional layering structure we believe these will make it difficult for the larvae to penetrate by pushing between the fibres but this has still to be confirmed by testing.

Fig. 13. Tyvek bags made up to size for herbarium sheets. WeLock clip used on short side (a) and Escal clip used on long side (b) of bags

As the herbarium sheets measure 445 x 285 mm and the folders in which they are kept measure 450 x 305 mm it is advisable to have a further support inside the bag to make it more 'rigid'. This can be either of Plastazote® or archival board (with the corners rounded). These are best cut to dimensions slightly larger (i.e. 5 mm extra on each side) than the herbarium folder. To prevent the sheets from slipping inside the bag, corner strips can be added, which can be made from Tyvek® tape. Two styles of bags were made up from one length of Tyvek®: one sealed along the longest length and one sealed along the shortest length to fit the herbarium sheets or folders (Fig. 13). The bags of course can be made to any dimension required, limited only by the width of the heat sealer jaws.

The open end of the bag needed a secure closure mechanism and tests on various types were undertaken. Escal® strips, successfully used with Escal® barrier film, are advertised as hermetic seals for temporary use (website www.cwaller.de) and WeLoc® clips (marketed as Klippits), originally designed for hospital use for sealing fluid bags, are used for sealing food bags (www.weloc.co.uk). Both therefore provide a tight seal through which insects would not be able to penetrate (D. Pinniger, pers. comm., 2006; Figs. 9, 13). The Escal® strips measure 500 mm so will fit either style of bag (i.e. whether heat sealed along the long or the short edge) but it is difficult to use and appears to weaken with repeated use. (Also once it has been used to seal a thick layer it cannot then be safely used to seal a thin layer.) The WeLoc® clip 200 PA special measures 220 mm, it is easy to use and reliable on re-use, but it is heavy in comparison to the Escal® strip. Unfortunately, it is limited by its size and could only be used with herbarium bags that were open at the short edge. Experiments with hook and loop tape (e.g. Velcro®) showed that it was difficult to attach the strips and there will inevitably be an area at the heat sealed edges where it may not be fully covered with the tape, but this will need further testing.

Another consideration is the cost of producing these bags.

Together with the insect trails, Phase 2 is to undertake practical experiments to show the durability and effectiveness of these bags.

Conclusion

From this preliminary investigation it was established that Tyvek® type 14 (43 gsm) can be heat sealed and has the potential as a short-term protective barrier for herbaria. Further investigation also needs to be undertaken to find other suitable clips to seal the bags effectively. Everything is now in place for the insect and durability under handling trials to go ahead.

In summary, the advantages of Tyvek® as a preventive conservation material are that: it is readily available; the bags are easy to make; it can be heat sealed using the Crossweld®; and it has museum-wide application. The disadvantages are: the weld is uneven; the length of weld is limited by the size of the

Crossweld®; and limited by the size of effective clips.

These bags, therefore, have the potential to be re-used but they are intended for short-term storage only.

Endnotes

1. PRE-MAL (Pest Research and Education – Museums, Archives and Libraries). 'Strategies in the battle against pest insects in collections' Symposium held at the Swedish Natural History Museum, Stockholm, September 26, 2005.
2. Dutch Nursery, Brookmans Park, Hertfordshire.

Acknowledgements

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Websites

AATA http://aata.getty.edu
BCIN http://www.bcin.ca
Conservation on Line http://palimpsest.stanford.edu
Museums, Libraries and Archives http://www.mla.gov.uk

Appendix: Suppliers

Crossweld® heat sealer
Preservation Equipment Ltd
Vinces Road

NatSCA study trip to Sweden – 19th / 20th May, 2006
- Kate Andrew, Wendy Atkinson, Steve Thompson

This year's Natsca trip was a smaller scale event than previous trips. Planning began at last year's SPNHC meeting in London – several Swedish delegates attended the meeting and were approached as possible contacts. Steve Thompson took on the organising, with details firming up as the group committed to going, but travel arrangements were made individually.

I think we were all rather worried by stories of how expensive Sweden was and wondered whether we would be able to afford to eat when we were there, that was partly why most people chose to stay in Uppsala. In the event, food, drink and accommodation seemed to be about the same as London prices and the airport to Stockholm link train was comparable in price to the Paddington-Heathrow Express. We stayed at a youth hostel in Stockholm and by paying a slightly higher amount got a 4-bed room to ourselves. We also bought a three-day Stockholm Card so got free travel on public transport and free admission to the many museums (more later)

I (KA) was enchanted by Stockholm, its built on a number of islands between a large inland lake and inlet of the Baltic sea, the original city (*Gamla Stan*) is mainly located on Stadsholmen and this island retains its cobbled streets and tall closely packed merchant's houses with alleys leading down to the quays. It was quite something to see the water pouring out of the lake under the bridges and people fishing for salmon (amongst other things) right in the centre of the city – equivalent to someone fishing off Waterloo bridge. During our stay, we visited six of the islands and travelled between them by metro, bus and short ferry trips, in addition to the medieval old town, the architecture is a mixture of modern high rise (in the central shopping area and residential areas), neoclassical and folk-influenced art deco, wide tree-lined streets, lots of parks, lots of public art and areas of allotments with little wooden summer houses.

The official part of the visit began on the Friday morning with an all day visit to the Naturhistoriska Riksmuseet. The Uppsala party had been guided in by a member of staff who lived in Uppsala. Fortunately, we ended up getting off the same metro train, otherwise we may have found it tricky to locate the botany building on the university campus, despite having been e-mailed a picture of it in advance. We were welcomed by a group of staff and served tea and coffee and cinnamon pastry, the group included Ingela Chef-Holmberg, whom I had first met in Cambridge teaching on a preventative conservation course, I knew several other people we met during the day from pest conferences and the SPNHC meeting, and it was good to see them in their home environment.

Pia Osetnsson and Cecilia Herbst explained the history of the Botany section. Linnaeus founded the organisation in 1739 as the Royal Swedish Academy of Sciences, although the present botany building was built in 1916. Linnaeus sent collectors out worldwide to collect plants and to try to propagate economic crops in Sweden in order to avoid having to import crops. We were fortunate to be shown the cabinets containing what remains of Linnaeus' original specimens (some 4,000) – his widow sold most of his collection to a Mr. Smith in England and this collection became that of the Linnaean Society in London. A collection made by Linnaeus' son has also remained with the museum. We also saw a herbarium of 1701 from Gotland, collected 40 years before Linnaeus and material for the 1878 North West passage expedition. The botanical collections now number some 9 million specimens and plans are underway for a new collections centre extension to the building, possibly to coincide with the 300th anniversary of Linnaeus' birth in 2007.

After this introduction, we were led on tours of the various sections of the building – Monika Myrdal showed us the Cryptogamic Botany section, Ove Johansson, the Palaeobotany (which amongst the 250,000 specimens we were surprised to find a considerable number of Jurassic plants from the Yorkshire coast plus some very impressive live plants) We then changed buildings, to the main building that houses the public galleries and were shown around the mineralogy section by Henrik Skogby, looking at amongst other

things, a collection of meteorites, mainly from Northern Sweden. The mineralogy collection contains some 150,000 specimens including 85 holotypes. Henrik took us from the stores into the mineralogy galleries via the staff entrance and we were all stunned by the contrast between typical museum store (wooden cabinets and drawers) and a glittering cabinet of curiosities style display of an historic collection, intricate parquet flooring, tall windows, lit by chandeliers with polished wooden cabinets with bevelled glass containing a very impressive array of mineral specimens. The more modern display contained some ingenious "open storage" style pull out units – like thin upright display cabinets, in which, as you pulled out a unit, the lighting switched on.

We then moved on to the basement area to meet Peter Mortensen to look at the bird collection. Peter explained that, since the 1970s, the law protects all Swedish wildlife, other than about 8 species, and any dead specimens have to be handed in to the police. Large specimens are autopsied by the vetinary college, smaller species come to the museum and are measured and sampled for environmental pollution and converted into study skins and osteological specimens, consequently, the collections are pretty comprehensive and in the case of woodpeckers, show clearly when the lesser and greater spotted woodpeckers became extinct in Sweden. Pest control in the collection is managed by freezing and desiccant dust is used under the cabinets.

The staff then joined us in the museum café for a rather good lunch. Much discussion had as to which species of fish we were eating and exactly why the sole of a shoe appeared to be the colloquial name for the beef stew.

After lunch, we visited the osteological store with Olavi Gronvall. This was a combination of old osteological mounts, grouped rather effectively together, and compactor units with osteological material grouped by species. Game heads with antlers are mounted on mesh panels, hanging form the ceiling and accessed via a pulley system, making the best use of a very high ceiling. There are about 75,000 specimens in the collection including the 78 mammal species present in Sweden, (for example, bears and wolves, seals and dolphins), but the collection also contained quite a lot of elephant material.

It was then off to the Entomology department for a tour by Niklas Jonsson. Here we moved through room after room of mainly new wooden cabinets containing entomology drawers, some rooms had mezzanines with material arranged around the balconies as well. The team is experimenting on the best style of drawer and pest control methods and is also planning to allocate unique catalogue numbers to every specimen. At one point we encountered Geoff Hancock (who joined us later), working in one of the lab areas, researching some of the older collections.

It was then time for a break, more cake and a explanation by Monika Akerlund of the award winning PREMAL (Pest Research and Education – Museums, Archives and Libraries), a preventative conservation programme led by the museum that involves staff from across the museum and across Sweden.

After thanking our hosts for a most enjoyable, interesting and well organised day, the group then had a chance to look at the galleries, visit the shop and for a couple of us, a quick visit to the Palaeontology department, currently merging their own and a recently donated large collection. At the end of the day, several of us joined staff for a seminar by Peter and Rosemary Grant describing their research on small-scale evolutionary changes on bill shape and song in a number of Darwin finches on one of the Galapagos Islands.

The group met up with Geoff and his wife and headed into Stockholm for the traditional aimless group wander looking for a suitable venue for a meal and a beer. We ended up in an Indian restaurant that, despite being on the touristy part of Gamla Stan, was quite cheap.

The Uppsala gang headed off for more natural history the next day, but we spent the next day and a half in Stockholm. I had long wanted to visit the Vasa warship – sunk on its maiden voyage in 1628 due to combination of top-heavy design and an innovative second gun deck, with the lower deck too close to the water line (aka Zeebrugge disaster). It was raised in a near-complete state in 1961, the brackish water means that *Teredo* ship "worm" cannot survive and so the wood was undamaged. A lengthy conservation project ensued and the ship moved to its present purpose built building in 1987. It is spectacular; by 2001, over 20 million people had visited it.

On Sunday morning, after locating the left luggage lockers at central station, we were able to fit in a very

quick visit to Skansen. This is a huge open-air museum on one of the islands that displays and interprets traditional buildings from all-over Sweden. We were entertained by musicians in a church playing traditional tunes on violins, the efficacy of the toothache tree was explained to us (and we saw the tree with lots of wooden toothpicks sticking out of the north side) and we saw unleavened bread being baked. All too soon it was time to make our way back to the airport.

For the rest of us, for the second day of the trip we stayed in Uppsala. This is a small, quiet and attractive town, about as far north of the airport as Stockholm is south, and so made an excellent base, considering that we had already intended to visit both places anyway. Our hotel was only fifteen minutes walk from the railway station, just as well considering that the taxi fare would have been extortionate. It was on the edge of the university area, very convenient for the museums we were down to visit, to say nothing of the other twenty or so museum sites in the town. While attention may now be focussed very much on Stockholm, Uppsala has been the cultural heart of Sweden for much of its history, and is still an important cultural centre. It is also the centre of attention for anyone interested in Linnaeus, being his home town and centre of operations. There are some twenty or more museums in the town, most of which there was not time to visit, and many of which are part of the university. Our main concerns were with the natural history museums and collections, of which there are four, including the Linnaeus house and garden.

The day started as grey and wet as we might have expected all along, although in fact the weather was fine for pretty well our entire trip. We took the ten-minute walk up to where the university museums were situated, around a large square on what is a very open university campus, almost deserted at this point in time. The former Botanical, Zoological and Palaeontological museums of the University combined in 1999 into what is now the Museum of Evolution. It took us a little while to find the correct entrance, but having been welcomed in, we found ourselves in a new Botany building. Magnus Liden, the curator of the Linnaeus Garden, who would later show us around that, and Mats Hjertson, of the Botany Department, who showed us around the department, met us. There is no Botany museum as such, but they have very extensive collections, and an active research programme.

Mats started off by showing us the pressing room, drying room and the freezer. The feeling was that this was an active department, judging from the amount of botanical material in the presses and also the shelves of papers drying in the drying room where the temperature is at a very warm 40°C. The walk-in freezer runs at -30°C which allows for whole cabinets to be put in there in one go, something they recently had to do when they had a pest outbreak in the fungi collections. They do not use sticky traps at Uppsala, but carry out regular visual inspections, which seemed to have served them well so far.

Next, we went on to the herbarium itself. It was lovely - very bright and airy and the collections were all housed in new wooden cabinets. Type specimens – including vascular plants, fungi and Palaeobotany items, are all housed separately in the 'type room' and there are 2 to 3,000 types. Mats told us that during the late 1800's – early 1900's there was a strong focus within the department on East African botany – in countries such as Kenya, Ethiopia, Tanzania, the Flora of Ethiopia having a strong Uppsala link. Today, staff from Uppsala are working on the Floras of Somalia, S.E. Asia and Africa.

The Botany collections amount to around 3 million specimens, with around 360,000 having been databased These are divided into 1,700,000 vascular plants, 250,000 bryophytes, 60,000 algae, 360,000 fungi and 500,000 lichens; one of the top lichen collections in the world. The collections date from 1785 when Carl Peter Thunberg (1743-1828) became Professor at Uppsala University. His collections from South Africa, Japan, Java and Ceylon, along with those of Burser and Celcius, are some of the most important botanical collections at the university. Thunberg was a great explorer and brought back thousands of specimens back with him, naming over 70 new genera and almost 2000 new species. We were next shown the 'Treasury', the room that holds the important collections of Thunberg, Celsius, and Burser. This was a real treat. The Celsius herbarium is contained in 8 volumes. Olaf Celsius the Elder, was Dean of Uppsala and the botanical authority there before Linnaeus who was later to often refer back to this herbarium during his own studies. Plants collected by Linnaeus are included in Celsius's 'Flora Uplandica'.

Joachim Burser was born in Germany and became professor of Botany at Sorö, Denmark. His 'Hortus Siccus' comprises 24 volumes of plants collected from middle Europe and Denmark. Burser's herbarium was

taken by the Swedes as spoils of war in 1660, and contains type specimens later described by Linnaeus. The majority of the room contained Thunberg's herbarium housed in his original grey-white wooden herbarium cabinets and comprises of 27764 specimens.

From the Botany department, we went over to the Zoology museum, which was not open to the public at this point in time. We began on the top floor, where they have set up a room in the roof space, with sloping ceilings, and strikingly lit, green on one side, blue in the middle and red on the other side. On the green side were a series of arctic and sub-arctic mammals and birds, and on the red side a series of skeletons of decidedly non arctic animals, including a series of flightless birds, some extinct, and culminating, via an elephant, in a human skeleton. In the centre was a large whale skeleton.

This room was in marked comparison to the main part of the museum, which turned out to be an absolute cabinet of curiosities, set out in what would in Britain be considered a Victorian fashion, with rank upon rank of tall glass cases filed in a broadly taxonomic arrangement. Some of these specimens were rather special, with a thylacine and a passenger pigeon amongst them, but perhaps the most bizarre were the large inflated organs of a number of large mammals. It wasn't entirely clear why these had been so treated but they were certainly eye-catching.

From the Zoology museum we went to what was for me the biggest surprise of the trip, the Palaeontology museum. One surprise is that an Englishman, John Peel, has curated it for the last twenty years but the biggest surprise was the largest collection of Chinese dinosaur material outside of China, which filled two large galleries in spectacular fashion. Given only twenty minutes in the museum, it was not clear just what else there might have been to see, but these on their own our worth visiting the museum for. They are currently undergoing a conservation project under the tender care of Caroline Buttler from the National Museum of Wales. This is a place I (ST) will certainly be aiming to revisit.

All too soon, it was time to move on again, to meet up with Eva Bjorn, one of our hosts for the afternoon. Lunch was taken in a very pleasant, and yet again not expensive, fish restaurant (not, I hasten to add, the glorified chippy it would be in most places in Britain) in the town centre. After this we headed to Linnaeus's House. Although we had been given to understand that a major restoration project was underway, this had, in fact, been delayed by two weeks, so that we could actually be taken into the house, a major bonus for us. Having slipped on our cute blue plastic booties, we were given a guided tour of the house, and a potted history of Linnaeus's life and work there. In fact, this is one of two places that Linnaeus used, the other, at Hammarby, a few miles out of Uppsala, being his main summer and work residence. Alas, time did not allow us to visit this as well. Next time, perhaps.

We finished the trip by being taken round the garden, in one corner of which the house is situated. The orangery at the back of the garden contains a small exhibition about the garden's history. The garden is laid out according to Linnaeus's own plan, and all the species grown are known to have been cultivated by Linnaeus himself.

This brief account of the trip gives only a limited idea of what there is to be found in Stockholm and Uppsala, and we can heartily recommend Sweden as a place to visit. Contrary to our expectations, it did not turn out to be horrendously expensive, no more so than London, certainly. In return, we found the people very friendly and helpful. Our many thanks must go to all the curators and other museum staff in the various institutions we visited, who were faultlessly welcoming and helpful. For my own part, I (ST) would be more than happy to set up another study visit, so if anyone is interested in going, well, you know where to find me.

Ethics and Authenticity in natural history exhibits: the public wants what the public gets

- William Lindsay, Royal College of Art

Notions of authenticity and objectivity underpin the aims of natural history displays in museums. These ideals present themselves at many levels, from the materiality of the objects presented to the explanatory narrative that they convey. However, public perception may not always reflect the realities of the message offered. This paper considers the extent to which these ideals are actually achieved and what this may mean for the public for whom museum professionals aspire to the highest ethics of investigation, exhibition and explanation.

Introduction

Susan Crane (2000), writes of her relationship to museums:

> *I don't enjoy visiting museums anymore, or at least not the way I did before I began to study them. Too close a proximity to the subject has produced a familiarity akin to contempt…The expectation of having a particular cultural experience prevents all but the random joy of discovering something as "new"…*

One museum that confounded her expectations is the Museum of Jurassic Technology (MJT), Los Angeles. With its display of "Protective Auditory Mimicry" illustrated by a beetle that has evolved to sound like a visually similar pebble, and the bat, *Myotis lucifugus*, that flies through walls by virtue of the frequency of its sound emission, this is a fantastic (in the true sense of the word) museum experience. Presented as an educational institution dedicated to the advancement of knowledge and the public appreciation of the Lower Jurassic, MJT's creator, David Wilson, comments

> *We're definitely interested in presenting phenomena that other natural history museums seem unwilling to present* (Weschler, 1995)

The experience of the MJT is confounding because it creates its own world within which it is disconcertingly consistent and authentic; the reality that seems out of place is that of the visitor.

Fantastic objects, such as dressed Mexican fleas, or *homo diluvii testis*, the supposed human witness to biblical flood (in fact a Miocene fossil salamander), are not uncommon in natural history museums. The fleas serve as whimsical self-parody to illustrate the seriousness of organized science, the salamander to illustrate the belief system that science has nullified; both give authenticity and legitimacy to the taxonomic and classificatory systems that govern understanding of natural history collections.

However, authenticity in natural history museum exhibits can be unclear to their audiences. The mastodon skull presented in The Natural History Museum, London (NHM) as a possible source of the myth of Cyclops prompts one couple's conversation:

> *What is it? Is it a dinosaur? What is it? Why has it only got one eye?*
> *- Well, it's a… it's, well, obviously it's only got one eye because half the face is missing.*

Authenticity and natural history

How is authenticity established in the display of natural history specimens? In art, particularly with regard to paintings, a range of sources are cited in support of authenticity: chemical composition of paint to indicate age and provenance, the structure of paint layers as a reference to the artist's technique. Or the opinion of a committee of experts, as in the case of the Rembrandt Research Project (RRP) and Liverpool's Walker Art Gallery, where authenticity and attribution come with a high impact on reputation, as well as finance:

> *The Walker Art Gallery's portrait of 'Rembrandt as a young man' is possibly not a self-portrait by*

> *Rembrandt… based solely on a comparison of seeming differences in brush-style… Prof. van de Wetering (chair of the RRP) himself admits that the RRP's view is solely an opinion and a changeable one, "which anybody can take or leave in cases where the arguments … concern no more than style and quality* "… (Walker Art Gallery, 2005)

In the context of natural history museums three aspects of authenticity seem most prominent:

a. authenticity in the object
b. authenticity in the narrative
c. authenticity in the experience

Authenticity in the object

Real has importance in a museum context. People like to see *real* things, we are concerned with the preservation of the *real* thing and conservation must guard against altering the *real* object. However, terms such as forgery and fake (Jones, 1994), replica and reconstruction, original and imitation, should not be taken at face value. It has long been a tradition in the curation, research and display of natural history that something other than original material composition can be valid. A copy of the Mona Lisa might not satisfy the viewing public but *Archaeopteryx*, arguably the single most important specimen among the 70 million specimens within the NHM, is commonly represented by a high quality glass fibre copy. In the world of art, market price as a vector of value would be recognized as a legitimate reason for putting a painting on display, as with the exhibition tour of the National Gallery's 'Madonna of the Pinks' (National Gallery, 2004). But, with *Archaeopteryx* valued somewhere between £1million – £10million, different standards must apply in the relationship between natural history museums and their public. The source of that difference is probably the same as that which defines our attitude to copies and imitations, and lies in issues of uniqueness and systematization and, perhaps fundamentally, in the notion that a natural history museum experience is a didactic experience and the museum chooses what is best for its public.

Hein (2000) described the conflicting ideals that have motivated museum collections as "a fascination with the unique", and "an interest in the universal… so that their [specimens] individual differences can be reconciled with their generic oneness". Uniqueness positions a specimen within an organizational system, but this also makes it a representative of other specimens. And if the specimen is only a representation, the messenger and not the message, perhaps that helps to accommodate other kinds of representations, such as three-dimensional replicas, illustrations, and computer visualizations.
The enforcement of truth that Muñoz Viñas (2005) identifies as a goal of classical theories of conservation is undermined in the study of natural history where a tradition of highly interventive investigation is difficult to reconcile with the conservation process in other fields. The alteration of a specimen by irreversible preparation for study, perhaps accompanied by the inclusion of replica parts indistinguishable to the eye, reinforces its authenticity and 'truth' ultimately by establishing or correcting its taxonomic position.

Natural history exhibitions are full of composite objects. Plastics, plaster, iron, synthetic hair, stuffing that gives shape to taxidermy specimens – all contribute to the fakes on show. And, although intended to deceive in order to present informative completeness, observers may still be disappointed to find out that the specimen is not 'real', even although in other cases they may assign greater legitimacy to reconstructions based on slim evidence.

Accuracy is not a prerequisite for authenticity in natural history museums; many knowingly incorrect reconstructions can be found on display, sometimes highlighted by the corrections that have taken place. The continued display of unalterable dinosaur reconstructions alongside those refashioned in the 1980s to lift their tailsin the 1980s means that the public must contend with the epistemology of dinosaur studies, as well as issues of ecological authenticity.

Authenticity in the narrative

The distinction between the natural history specimen as representative of a group and as a unique individual may not be clear in a museum's overall approach to the narrative it offers. Didacticism is the intention, but 'internal coherence' is sometimes lacking (Hein, 2000). Orphaned specimens may be displayed, disconnected from the narrative around them. As a consequence they may take on iconic status (Alberti, 2005)

through deliberate intention, or by popular acclaim, as with the walrus at the Horniman Museum (2006) or the Blue Whale model at the NHM, of through the lack of connection.

Objects do not tell their own story, especially when they become icons by default. When the narrative is the story of life and its diversity, the exhibition of natural history specimens is problematic; the one thing that qualifies a specimen to illustrate life is the one thing that they are singularly lacking - life.

The reconstructed plaster and metal skeleton of the giant sloth, *Megatherium*, isolated in gallery 30 of the NHM is an example. Visitors are drawn to it, partly because it is unexpected. It plays no part in the narrative presented by the reptiles on display alongside. But the museum is known for its dinosaur displays, marketed repeatedly as the essence of a 'good day out' and promoted as icons. Unsurprisingly, visitors can be overheard referring to *Megatherium* as a dinosaur, as they pose for their souvenir photos. 'Large animal skeleton', has come to mean 'dinosaur', just as 'hoover' means 'vacuum cleaner'. In part, *Megatherium* is where it is because it did not fit into any exhibition scheme and cannot be dismantled without causing much damage.

Authenticity in Experience

The exhibition of natural history specimens to enhance learning and understanding confers a particular legitimacy and authenticity on them. But while the learning experience promotes one value other experiences may confuse this. Hein (2000), speaking of newer approaches to display, states

> *Everyone loves dinosaurs…but if numbers are a clue, museum visitors are happy with cleverly engineered models that roar and move as with the carefully researched and reassembled paleobiological specimens found in traditional natural history museums.*

And she notes that design and spectacle appear as central elements, sometimes pre-empting narrative order for dramatic delivery. However, narrative order is not only pre-empted by spectacle, but spectacle can also undermine authenticity. Numbers are not themselves a measure of quality.

Animatronic dinosaurs are the preeminent *dramatis personae* of the museum 'great day out'. Elsewhere experiences such as Weald & Downland Museum or Colonial Williamsburg place authenticity of object and accuracy of use at the centre of their aims to give experience of history and custom. Beneath the theatricals and boiled sweets, the aim is to convey knowledge rather than to create what Hein (2000) calls, the "self-vindicating' experience of the theme park. But there cannot be authenticity in the experience of a human encounter with a dinosaur and, anyway, what experience do we wish the public to draw on to make sense of this?

The diversity of messages now on offer in natural history museums can be seen as the antidote to the museum experience of yesteryear. But in the discordance created, this diversity may not be understood and the 'experience' of fantasy may distract from the rest.

Ethical perspectives

Since the objects displayed in natural history museums are not necessarily real but may be presented as such, since they may disrupt and conflict with the intention to educate, and since the learning experience may be reduced to a self-fulfilling TV reality show, the ethical dimension seems relevant.

Many museum organisations promulgate codes of ethics e.g. the International Council of Museums (ICOM, 2006), European Confederation of Conservator-Restorers' Organisations (E.C.C.O., 2002), and the Curators Committee of the American Association of Museums (Curcom 1996); to be professional is, apparently, to be ethical. Even the USA's Central Intelligence Agency has an ethics programme, for which it was awarded the Outstanding Ethics Program Award from the USA's Office of Government Ethics. Clearly what is ethical - and professional – is not absolute or universal, and while "Ethics is about how we ought to live" (Singer, 1994), 'how' and 'ought' allow considerable room for manoeuvre. Edson's (1997) view that public trust is the basis of museums, and upholding this is an ethical concern, is advanced by Sola (1997) when he writes that the ICOM code of Professional Ethics could go beyond functional concerns by 'stating

that truth should be the basis of any message or image used'.

Museums do engage with the public on ethical issues. For example, they may justify their use of incomplete or damaged specimens from the perspective of an ethical balance sheet. Drawing attention to the damage that has occurred in the museum to the displayed taxidermy specimen, the museum expresses its concern for wildlife conservation by rejecting the collection of a new specimen; no recently living creature was killed in the making of this display

Conclusion

Assumptions are made about museums representing science: that they are truthful about their objects and that these represent something authentic in their material, their message, and their experience. Perhaps this is why the Museum of Jurassic Technology is so disconcerting; its authenticity comes from the sincerity with which it welcomes the assumptions of its visitors.

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Overview of the NatSCA Biochemistry Seminar
Simon Moore, Course Coordinator

Kew Gardens Jodrell Laboratory
9.11. 2006

The theme of the day largely concerned the biochemical make-up of our specimens and what can happen to them, at a molecular level, under certain conditions.

The first two talks concerned a brush-up of biochemistry and terminology for those of use who were less-experienced or who had not partaken since 'A' level or University days!
Simon Moore introduced the seminar to some basic facts about organic molecular structure, bonding, cyclic structuring and amino acids which led directly into a more detailed continuation by David Lampard (Dundee) who led us further through the structures of proteins, including secondary and tertiary 'foldings', sugars and other carbohydrates, enzymes and all the other relevant building blocks of biochemistry.

Following a quick break to refresh our already-boggling brain cells, Amandine Pequignot (Paris NHM) outlined the molecular processes that occur with fluid preservation. This naturally included the fluid fixation process that prevents protein breakdown into component amino acids by cross-linking them and coagulating cell contents but still preserving the methylene –CH2 bridges that bond proteins together. We should always use formalin or formalin-based fixatives and then transfer specimens to alcohol/s if required. IMS is really useless as a fixative since it simply displaces –OH ions in proteins and reduces inter-molecular spaces leading to stiffness. On the other hand if used as a storage medium, it will slow down or prevent harmful hydrolysis. She mentioned the differences between multi-layer water within proteins which is weakly bonded by -H- bonding and van der Waal's bonds, as opposed to bond water which, if removed, reduces the gaps between molecular layers and leads to specimen rigidity.

She also re-used some of her talk from the NatSCA/SPNHC conference (2005) about comparing the tensile strength of mammal skins fixed in various fluids including propan 2-ol (isopropyl alcohol), concluding that we should be using 5% formalin (2% formaldehyde) and that the normal fixation strength can lead to too much rigidity in (mammal) specimens. Another interesting conclusion related to rehydration which was found to be easier from alcohol pseudo-fixation than from formalin fixation.

Carrying on this theme of fixation, Jules Carter then treated us to the latest findings in DNA fluid preservation. He disfavoured the use of Steedman's 1979 fixative as suitable for larger specimens – it was after all, devised for zooplankton – although this author still finds it useful for certain macro-invertebrates. Concerning the continuing debate about formalin scrambling or interfering with DNA, he informed us that formalin-fixed DNA is perfectly OK but would require extraction through critical point drying. Alcohol fixation may release denaturants leading to breakdown of DNA structure. Many specimens exude certain compounds during fixation, extracted by the fixative and although these may colour or appear to contaminate the fluid they will reach an equilibrium. Changing the fixing or preserving fluid will upset this equilibrium and could bring about further extractions from tissues but that certain tannins and resins, extracted during plant fixation, and which also contaminate the fluid, can intefere with plant DNA and would therefore need to be removed. Ethyl acetate, used as a killing vapour for entomological specimens, is disastrous for DNA extraction – the reason is presently unknown.

He also added to the alcohol fixation problem since IMS and ethanol will denature and aggregate proteins and cause disruption to tertiary hydrophobic bonding which results in steric arrangement and produces isomers (mirror image molecular structures) which may have entirely different properties. Dilution of alcohols will initiate further hydrolysis due to added amounts of water which is bad for specimen tissue stability.
In conclusion, to preserve DNA effectively:
Cryo- or low temperature freezers maintaining tissues at -80°C, or storage in liquid nitrogen but requires expensive and dedicated equipment.

Drying – Critical Point Drying using acetone or absolute ethanol, or freeze-drying followed by storage in a cool and dry environment.

Finally: rehydration removes vital chemical elements through detergency action and must be only be carried out for gross anatomy tissues and specimens, otherwise leave the tissue in a dry state.

Some may remember Bob Stoddart from Velson Horie's Manchester Museum fluid preservation seminar in 1989. He gave 3 presentations showing the basic structure and compounds involved in biological museum specimens and how, under changing conditions, these vital compounds can become altered, whether by breakage or shortening of a molecular chain at some vital and 'vulnerable' point, and how other compounds (some deteriorative) might be formed as by-products. These facts he linked to DNA structure and to medical and histological radical facts and it made me wonder how on earth we have managed to steer our collections (hopefully) through all these biological minefields over time!

His first talk comprised deterioration of plant specimens in collections, at first outlining the importance of cell wall structure, reminding us of the primary layer comprising rhamnose and galacturonic acid, a secondary matrix of a different mix of carbohydrates and, for woody stems, a tertiary and impermeable layer of lignin.

He also reminded us about the composition of cellulose as a β-1,4, glucan except that it contained the carbohydrate mannose giving rise to gluco-mannan fibrils, also the composition and relevance of pectins, some of whose structures are so huge that they have not yet been 'mapped'! He explained about the physical versatility of carbohydrate complexes – the toughness of ivory nuts containing a rigid form of gluco-mannan against a similar structure in the root slime of a certain lily which has the opposite physical properties. He continued in similar vein outlining the components of other plant structures in our care and then switched to the effect that herbarium drying had on these components. How the removal of structural water causes woody structures to crack along medullary rays but that less lignified tissues can cope with this effect without breakdown. Fluid-preservation can be fine except that water exchange between cells and the preservative can occur and that complex molecules can expand into large gelatinous deposits. Lipids are extracted in IMS and lipocyte membranes can become degraded but are stable in dry form. Nonetheless, alcohol preserves gross tissues quite well except for algae which live in water and therefore require a more aqueous preservative.

He concluded with another word of caution relating to DNA, that it is vulnerable to photo dimerism in sunlight but that a glass barrier of more than 257 nm will be sufficient to absorb any harmful effect.

Adrian Doyle, ably assisted by Karen Roux from Thermolignum outlined the benefits of the Thermolignum process and how it compares favourably with other pest eradication processes by maintaining RH (within a 10% total-fluctuation parameter) and other environmental-relevant equilibria and guarantees a 100% kill over 24 hours. Invasive pests of museum objects are killed by 3 hours at 55°C as opposed to -30°C for 72 hours. Polymers can become 'unlaced' by freezing or non-equilibrated heating through their thermoplasticity properties. DNA is affected by exposure to naphthalene but is unaffected by the Thermolignum process. There were some warnings about the Thermolignum process. Phthalate-based adhesives can become yellowed by the process. A tight rebate on a taxidermy case can cause (old) glass to crack and may require a slower temperature increase and decrease to prevent this.

He concluded with a warning about the cumulative effect of varying pesticides and how these can affect specimen stability (Vicky Purewal is researching this presently).

Bob Stoddart then warned us about the effects that omnipresent airborne fungal spores can have on human physiology. He explained how hospital patients could mysteriously deteriorate and die due to cryptic fungal infections, he continued by showing fungal temporary surface infections as opposed to invasive - fungal hyphae actually spreading through tissues - demonstrated at a histological level using Gridley's Feulgen reaction or Grocott's silver impregnation. He also warned of the possible grave consequences of inhaling particles from dried pigeon droppings or receiving mammals specimens from California which might carry *Coccidioides* sporangia. After this gloom and doom which had us thoroughly washing our hands before the next tea break, he outlined how fungi produce digestive hydrolases and that some, such as *Aspergillus niger* contains a cocktail of 6 enzymes that can cause rapid tissue breakdown.

For his final presentation, Bob talked about pigments and how they can break down in museum collections through various agents. He started by explaining some light / colour physics and how iridescence and interference colours are produced through micro-ridged or micro-layered surfaces, e.g. butterfly wing scales. He

went on to explain how pigments are molecularly structured, linked through a single and double-bond alternating linkage or chromophore and how this can be shortened by photonic weakening of one of the double-bonded linkages and causing a reduction in colouration (eg. fading) and how a lengthening of the chromophore causes darkening. He mentioned the carotenoids as examples and how the various forms of carotene (α- β- γ- δ- &c) had slightly longer or shorter chomophores and therefore showed different colours. He showed how the blood pigmenting agent Haem – an iron-based tetrapyrrole (with carboxyl groups) can change its composition out of the bloodstream as it loses the iron ion and changes to biliverdin and bilirubin which explains how bruises change colour. He also showed how the magnesium tetrapyrrole, more familiar as chlorophyll, can similarly lose its magnesium ion to change to phaeophytin A and B becoming brown which is why marine brown algae, need only a small amount of sunlight to photosythesize, hence the kelp niche largely in the lowest intertidal zone. Another example is of porphyrins (found in deep sea jellyfish) which are dissolved by alkalis by removing the iron ion, just as acidified acetone removes haem from haemoglobin. He then ventured into anthocyanins and the families of phenolic –OH (hydroxyl) groups that react with amines and iron, especially ferric iron, to produce very strong pigments but how phenolics are prone to fading through oxidation. He told us how a strongly-pigmented flower can change from bright pink to green or blue when a burning cigarette, combining heat and tobacco smoke, is held very close. He also mentioned the litmus test using anthocyanins and how the red oxonium and the blue (basic) quinone colour reactions are reversible.

He then talked about xanthopterin in insects (with other pterins) and how these too can change colour through oxidation, also how lignin will oxidise in sunlight causing furniture to fade. Fungi contain polyalkynes with triple-bonded carbon atoms but that these triple bonds are amazingly weak and can easily break causing fading, something I have found with some, but not all, of the freeze-dried Hampshire fungal collection.

Papers Given at the Biochemistry Seminar, Jodrell Lab, Kew 9.11.2006

Basic Biochemicals
- David Lampard

Biochemistry is the branch of organic chemistry that deals with the molecules involved in biological processes and structures. Before describing some of the main groups of biochemicals it is worth defining some of the terms most frequently used when describing biochemicals and their reactions

There are three types of chemical bond that connect atoms and molecules together;

1. **Covalent Bond**, formed by the sharing of one or more electrons, especially pairs of electrons, between atoms.
2. **Ionic Bond**, formed by two ions with opposite charges, characteristic of salts. Also called *electrovalent bond*.
3. **Hydrogen Bond**, a hydrogen atom of one molecule is attracted to an electronegative atom, especially a nitrogen, oxygen, or fluorine atom, usually of another molecule.

Covalent bonds between carbon atoms help form the long chains of biochemicals.
Hydrogen bonds are often involved in determining the shape of large molecules

Displaying formulae

A molecular formula shows the relative abundance of the atoms more electrons, especially pairs of elec-

trons, between atoms.

Structural formulae show the relative position of atoms and bonds.

ISOMERS
Isomers are chemicals with the same molecular formula, but different structural formulae.

Biochemicals exist in right handed and left handed forms called dextro and levo, d and l. usually only the d forms are biochemically active.

Some groupings of atoms in a molecule have characteristic reactions independent of the rest of the molecule. These groupings are called **functional groups**.

Formula	Functional group	Comments
C=O	carbonyl	A carbonyl group on a non-terminal carbon atom is called a ketone group
CH	alkyl	
C=HO	aldehyde	
C=C	alkene	
-OH	hydroxyl (alcohol)	
-COOH	carboxylic acid	
-COOR	ester	'R' stands for any alkyl group
$-NH_2$	amine	

FAMILIES OF BIOCHEMICALS

SACCHARIDES are also known as carbohydrates or sugars
Monosaccharides, or simple sugars e.g., glucose
Disaccharides, polymers of two monosaccharides e.g. maltose, lactose
Polysaccharides, long chain polymers of monosaccharides e.g. cellulose, starch

LIPIDS Fats, oils, waxes etc.

Fatty acids and glycerides

AMINO ACIDS are the building blocks of polypeptides and proteins.

Saccharides or carbohydrates are:
- A source of energy for the body e.g. glucose and a store of energy, e.g. starch in plants
- Building blocks for polysaccharides (giant carbohydrates), e.g. cellulose in plants and glycogen in the human body.
- Components of other molecules e.g. nucleic acids, glycolipids, glycoproteins, Adenosine triphosphate.

Monosaccharides
Have the general formula (CH2O)n where n usually equals 3,5 or 6

Monosacccharides can be classified according to the number of carbon atoms in the molecule:

n = 3 **trioses**, have the formula C3H6O3 e.g. glyceraldehyde
n = 5 **pentoses**, have the formula C5H10O5 e.g. ribose and deoxyribose

('pent' indicates 5)

n = 6 **hexoses**, have the formula C6H12O6 e.g. fructose, glucose and galactose
('hex' indicates 6)

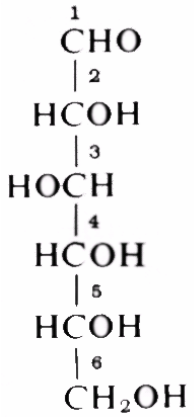


Fig.1 numbering of carbon atoms in glucose

When describing reactions in sugars the carbon atoms are numbered as above. All of the C6 sugars are isomers.

Monosaccharides are formed in metabolic pathways from simpler compounds with fewer carbon atoms. All the sugars formed from glyceraldehyde e.g. ribose, glucose, galactose, mannose are called aldoses because the functional group is an aldeyde C=HO. Aldoses are also known as reducing sugars.

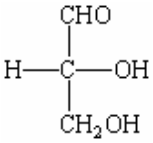
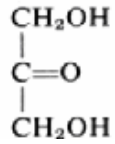
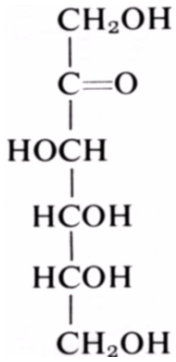


Fig.2 structure of glyceraldehyde

However sugars derived from dihydroxyacetone such as fructose are called ketoses because the functional group is a ketone C=O



Dihydroxyacetone



d fructose

Fig.3 formation of ketose sugars from dihydroxyacetone.

If the carbon chain is long enough, the hydroxyl group at one end of a monosaccharide can react with the carbonyl group at the other end to form a cyclic compound. When a six-membered ring is formed, the product of this reaction is called a pyranose ring.

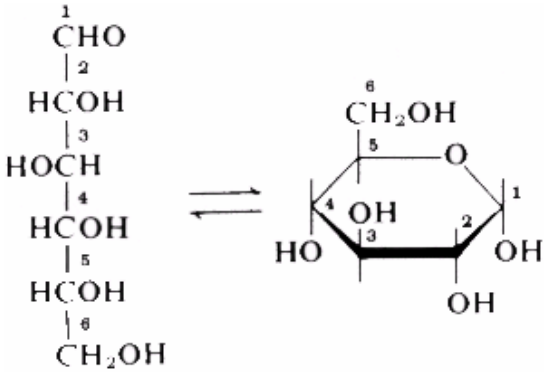


Fig. 4 equilibrium between chain and ring form of glucose

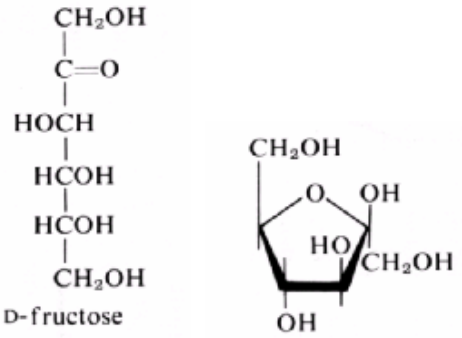


Fig. 5 chain and ring forms of fructose
Fructose forms a five sided ring called a furanose ring

A sugar may transform from the straight chain to the ring structure in an equilibrium state.
There are two forms of the pyranose ring named after the position of the hydroxyl group on the first carbon atom.

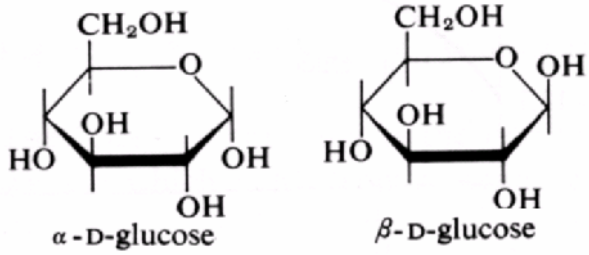
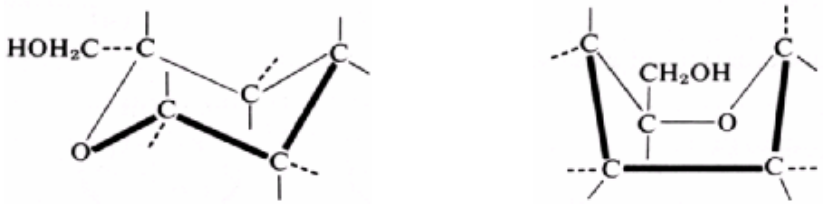


Fig. 6 the two positions of the hydroxyl group in glucose

The nature of the bonds in the ring configuration also means that the ring structure is three dimensional. In the ring configuration there are two shapes, known as the chair form and the boat form. The sugar is constantly changing between forms.



Chair form Boat form

Fig. 7 three dimensional forms of the glucose ring

DISACCHARIDES

The reactive groups on the carbon atoms make it possible for monosaccharides to react together to form polymers, long chain molecules consisting of repeating units. In sugars polymerisation takes place only in the ring configuration

The simplest polymers are DISACCHARIDES, the three most common are

maltose = glucose + glucose

lactose = glucose + galactose

sucrose = glucose + fructose

Lactose and maltose are also reducing sugars because they are derived from aldose sugars.

Disaccharides are more common than simple sugars but cannot pass through cell walls.
Disaccharides are formed through a reaction that is called a condensation reaction, one which releases a water molecule. The resultant bond is called a glycoside bond. This takes energy to form.

The type of polymer formed is described according to the carbon atoms involved and the configuration of the monosaccharides.

Maltose for example has a 1,4, bond in the alpha position. I.e. the hydroxyl group on the first carbon of the first glucose molecule reacts with the hydroxyl group on the fourth carbon of the second glucose.

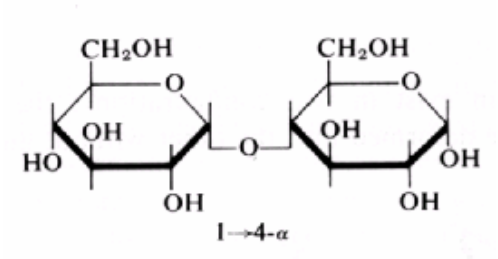


Fig. 8 Maltose showing the 1-4 alpha glycoside bond

The reverse reaction to split the bond is called hydrolysis and releases energy.

POLYSACCHARRIDES
Monosaccharides can undergo a series of condensation reactions, adding one unit after another to the chain until very large molecules (polysaccharides) are formed. This is called condensation polymerisation, and the individual building blocks are called monomers. The properties of a polysaccharide molecule depend on:

It's length (though they are usually very long)
The extent of any branching (addition of units to the side of the chain rather than one of its ends)
Any folding, which results in a more compact molecule
Whether the chain is 'straight' or 'coiled'

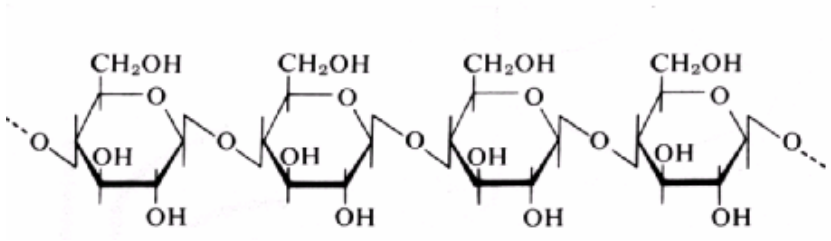


Fig. 9 cellulose showing glycoside bonds

Cellulose is formed by units of glucose, joined by 1-4 beta glycoside bonds.

Another common polysaccharide, starch, consists of a mixture of amylose and amylopectin

Amylose is a straight chain molecule of linked glucose units linked by 1-4 alpha glycoside bonds.

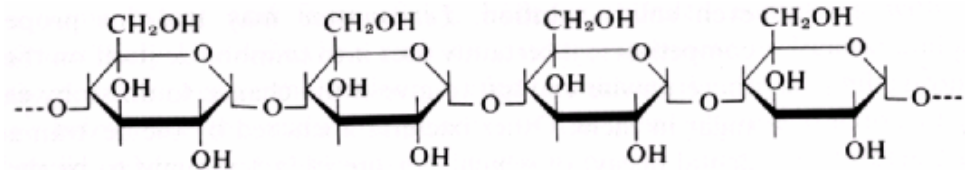


Fig. 10 amylose

The chain configures into coils.

Amylopectin is also made of glucose units but branches every 24 to 30 units

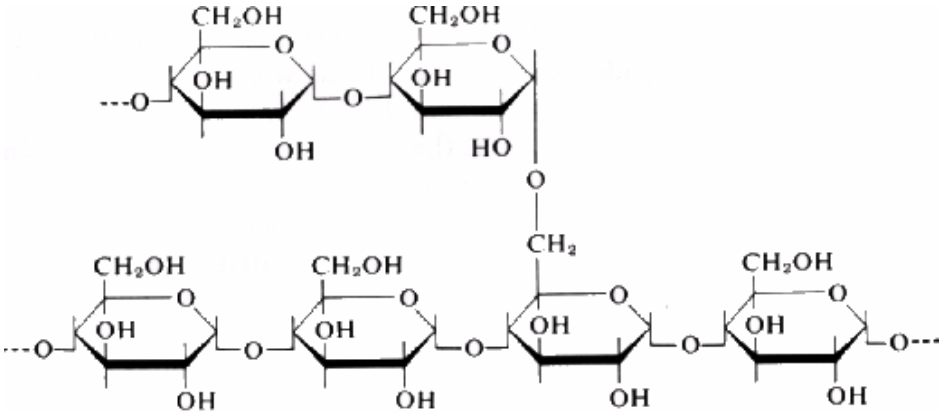


Fig. 11 amylopectin

In animals glycogen is the equivalent of amylopectin, however it branches every 8 -12 units

Chitin is a more complex polymer of glucose. It is constructed from units of N-acetylglucosamine (more completely, N-acetyl-D-glucos-2-amine).
These are linked together in 1-4- β fashion (in a similar manner to the glucose units which form cellulose).

In effect chitin may be described as cellulose with one hydroxyl group on each monomer replaced by an acetylamine group.

LIPIDS

The collective name for fats, oils, waxes and fat like molecules i.e. steroids.

Their roles include:
- components of cell membranes (phospholipids and cholesterol)
- energy stores
- chemical messengers (steroid 'hormones')
- protection, waterproofing, insulation and buoyancy agents.

The basic building blocks of lipids are a glyceride molecule, formed from glycerol (propane 1,2,3, triol) and fatty acids.

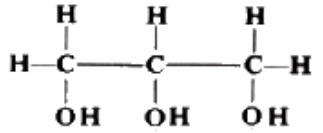
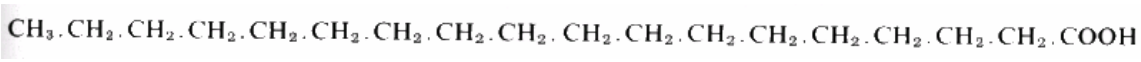


Fig. 12 Glycerol

Fatty acids are simple long chain hydrocarbons with a terminal carboxylic acid.



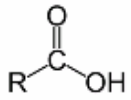


Fig. 13 structure of terminal carboxylic acid group where R is the rest of the chain

If all the carbon–carbon covalent bonds are single then the fatty acid is saturated. If some carbon-carbon covalent bonds are double bonds then the acid is unsaturated.

Triglycerides formed from saturated fats tend to be solid at room temperature, fats

Triglycerides formed from unsaturated fats are often liquid at room temperature, oils

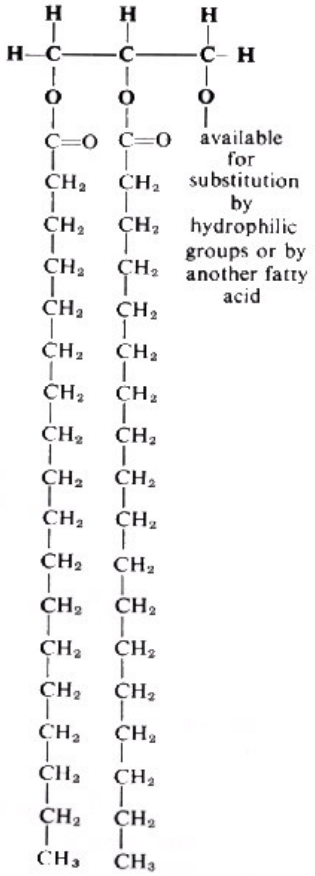


Fig. 14 structure of diglyceride

The glycerol molecule has three reactive alcohol groups.

If **one** fatty acid chain attaches a monoglyceride is formed. If **two** a diglcyeride If **three** a triglyceride

The reaction is a condensation reaction between a hydroxyl group of glycerine and a hydroxyl group of the fatty acid releasing water, the resultant compound is an ester. In the resultant glyceride, the glycerol component is hydrophilic and the fatty acid component is hydrophobic.

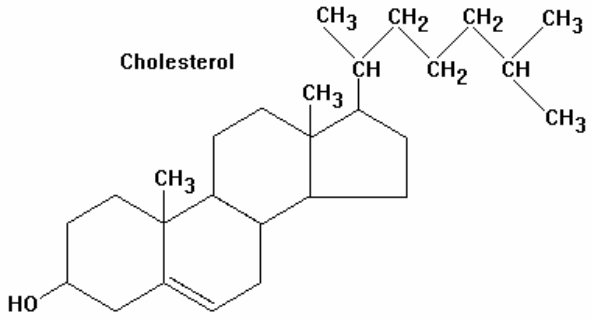


Fig. 15 Cholesterol showing the steroid unit structure

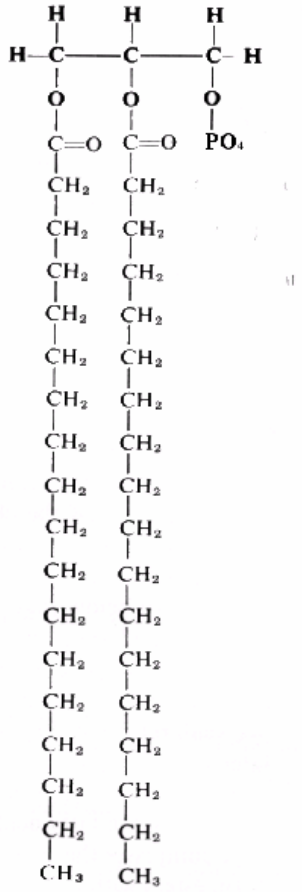


Fig. 16 phosphlipid structure

If one of the glycerol groups reacts with phosphoric acid the result is a phospholipid. Phospholipids are an important component of cell membranes. There are a number of lipids based around structure consisting of three six carbon and one five carbon fused rings, the steroids. One of these is cholesterol.

PROTEINS

Are naturally occurring **polypeptides**. They:

- contribute to the mechanical structure of animals, including humans, e.g. keratin in hair and fingernails, and fibrous proteins such as collagen in tendons
- enable animals to move, e.g. myosin in muscle
- facilitate transport of smaller molecules around animals' bodies, e.g. haemoglobin
- control the types and rates of chemical reactions in living things; then they are called **enzymes**, e.g. amylase

- are important components of the human immune system, e.g. immunoglobins

AMINO ACIDS

- Amino acids are the building blocks (monomers) of proteins.
- Amino acids have the general structural molecular formula NH2CHRCOOH.
- They have two important functional groups (regardless of the rest of the molecule):
- A carboxylic acid group-COOH and an amide group-NH_2
- There are twenty different amino acids used to make protein in the human body. Of these nine are called **essential** (meaning they can only be obtained from the food we eat) and eleven are **non-essential** (they may be synthesised in the body though they are usually obtained from food).

Essential amino acids		
Name	Abbreviation	Linear Structure
Histidine	**his** H	NH-CH=N-CH=C-CH2-CH(NH2)-COOH
Isoleucine	**ile** I	CH3-CH2-CH(CH3)-CH(NH2)-COOH
Leucine	**leu** L	(CH3)2-CH-CH2-CH(NH2)-COOH
Lysine	**lys** K	H2N-(CH2)4-CH(NH2)-COOH
Methionine	**Met** M	CH3-S-(CH2)2-CH(NH2)-COOH
Phenylalanine	**Phe** F	Ph-CH2-CH(NH2)-COOH
Threonine	**thr** T	CH3-CH(OH)-CH(NH2)-COOH
Tryptophan	**trp** W	Ph-NH-CH=C-CH2-CH(NH2)-COOH
Valine	**val** V	(CH3)2-CH-CH(NH2)-COOH

Non-essential amino acids		
Name	Abbreviation	Linear Structure
Alanine	**ala** A	CH3-CH(NH2)-COOH
Arginine	**arg** R	HN=C(NH2)-NH-(CH2)3-CH(NH2)-COOH
Asparagine	**asn** N	H2N-CO-CH2-CH(NH2)-COOH
Aspartic Acid	**asp** D	HOOC-CH2-CH(NH2)-COOH
Cysteine	**cys** C	HS-CH2-CH(NH2)-COOH
Glutamic Acid	**glu** E	HOOC-(CH2)2-CH(NH2)-COOH
Glutamine	**gln** Q	H2N-CO-(CH2)2-CH(NH2)-COOH
Glycine	**gly** G	NH2-CH2-COOH
Proline	**pro** P	NH-(CH2)3-CH-COOH
Serine	**ser** S	HO-CH2-CH(NH2)-COOH
Tyrosine	**tyr** Y	HO-Ph-CH2-CH(NH2)-COOH

Peptides and polypeptides

Two amino acids can undergo a condensation reaction to form a **dipeptide**.
The carboxyl group of one reacts with the amide group of the other, producing water. The amino acid units are linked by **peptide bonds** (sometimes called **peptide links**).

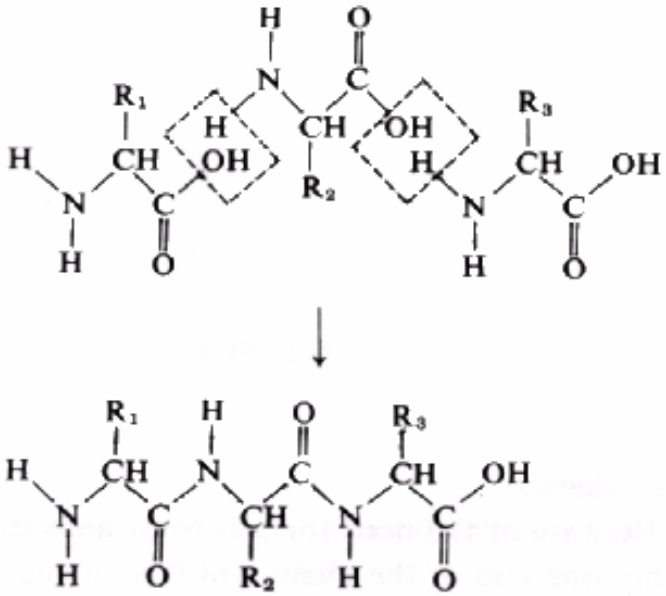


Fig 17. Formation of peptide bond

The addition of more amino acids through further condensation reactions result in a polypeptide. Long chain polypeptides are called proteins.

Protein Structure
Each protein has a characteristic sequence of amino acids in the chain. This sequence is called the primary structure. Further bonding can happen within a protein molecule (**intramolecular bonding**) and between protein molecules (**intermolecular bonding**). These bonds cause the molecules to adopt a three dimensional shape. Three types of bonds are involved, hydrogen bonds, covalent bonds and ionic bonds. Protein chains arrange themselves to maximise intra- and intermolecular bonding. This structure, when protein chains are held in place is called the **secondary structure**. This may be helical, e.g. keratin (the protein found in hair), or a pleated sheet, e.g. fibroin (the protein found in silk). These structures are held in place by hydrogen bonds.

The hydrogen atom of one peptide link is attracted to the oxygen of an adjacent peptide link.

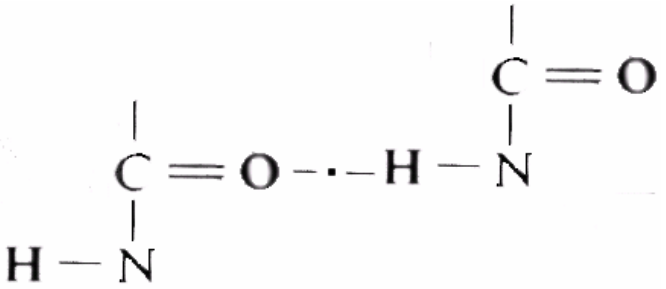


Fig. 18 Hydrogen bond formed between a carbonyl group and hydrogen from amine in adjacent protein chain

There may be further folding of protein chains e.g. into a globular shape.

This is the **tertiary structure** of a protein. Globular proteins include enzymes and immunoglobins. The structures are held in place by hydrogen bonds, disulfide bridges (sulphur – sulphur covalent bonds) and ionic bonds.

The precise structure of a globular protein is the key to specificity of enzymes. Similarly proteins that act as receptor sites on the cell surface can recognise specific molecules because of their shapes.

Finally proteins that contain more than one chain have a **quaternary structure**.

Examples are insulin and haemoglobin.

METABOLIC PATHWAYS
The formation and destruction of biochemicals takes place in metabolic pathways with the reactions being catalysed by enzymes.
Anabolism is biochemical processes involved in construction
I.e. the formation of complex molecules from simple ones
Anabolic processes require energy.
Catabolic processes involve the breakdown of larger molecules to smaller ones
They release energy.
Most biochemical metabolism take place in well defined sequences of reactions called metabolic pathways

Some important metabolic pathways
glycolysis – oxidation of glucose in order to obtain Adenosine triphosphate and producing pyruvic acid

citric acid cycle also called the **Krebs' cycle** or **tricarboxylic acid cycle**. part of respiration involving pyruvic acid eventually producing energy and releasing carbon dioxide.

oxidative phosphorylation – final part of respiration, disposal of the electrons released by glycolysis and citric acid cycle. Much of the energy released in this process can be stored as ATP.

pentose phosphate pathway - synthesis of pentoses (used in nucleic acids) from glucose and release of the electrons needed for anabolic reactions.

urea cycle - disposal of toxic ammonia into urea.

fatty acid b-oxidation - fatty acids breakdown into acetyl-coenzyme A, to be used by the Krebs' cycle. Coenzyme A is an important molecule involved in chemical reactions

Gluconeogenesis - glucose synthesis from smaller precursors, to be used by the brain.

Photosynthesis synthesis of glucose from carbon dioxide and water.

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Comprehensible biochemistry. Yudkin, M. Offord, R.

Chemistry for Biologists http://www.chemsoc.org/networks/learnnet/cfb/index.htm

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The Structures of Plant Tissues and the Effects of Drying and Fluid Preservation Upon Them

- Bob Stoddart

1. A CAVEAT

Biochemical knowledge, both of plants and animals, has largely been obtained, in the first instance, by the detailed analysis of a limited range of tissues from a few convenient and readily procured species. Comparisons with further tissues and species have then followed, but there is still a general limitation on the range of species and tissues analysed. This is a problem when considering the biochemical changes that may occur in natural history collections during their preparation and storage, for they will contain specimens for which biochemical analyses *in vivo*, or immediately after tissue death have never been performed and for which extrapolation from other species may be misleading. This is certainly the case for botanical specimens, where information is strongly biased towards higher plants, terrestrial plants, species easily obtained in bulk and species of commercial importance. Much more is known about the macromolecules in the walls and cells of plants important to the foodstuffs and the pulp and paper industries, for example, than is known of their counterparts in many algae, bryophytes or inconspicuous and non-commercial wild flowers. What follows largely refers to the tissues of terrestrial plants and algae of the *Characeae* and is unavoidably based upon analyses of a restricted range of species and their tissues. Hence xerophytes and higher plants adapted for, for example, life immersed in water, which are seriously under-represented in biochemical analyses, may have important differences from the 'average' in the properties of their tissues and the lower algae are certainly very substantially different from higher plants.

2. CELL WALL STRUCTURE: GENERAL

Plant cells have walls, which are domains of extracellular matrix surrounding the individual cells and under their control. The properties of the cell walls largely determine the mechanical properties of plant tissues and will preserve much of the shape and appearance of the tissue, even after the cells are dead. Thus the gross appearance and much of the histological structure of a specimen of plant tissue will be preserved in its cell walls and these walls will also provide a protective environment for the nucleic acids and proteins of the cells within and may limit leaching of some of the lipid.

Most of the dry mass of a 'typical' plant cell wall is attributable to carbohydrate, either in the form of polysaccharide or glycoprotein glycans. The rest of the dry mass derives mostly from protein, almost entirely as glycoprotein, mineral, chiefly calcium salts and silicates, and a variable amount of plant phenolics in the form of lignin. Other components are minor. In life, however, water can be a substantial proportion of the mass of a plant cell wall, especially if the wall is physiologically in a highly aqueous environment.

3. CELL WALL MOLECULES

Terrestrial plants and the higher algae have walls which are usually described as being composed of cellulose, hemicellulose and pectins, together with minerals and lignin. These fractions are defined by the methods of their extraction and none of them consists of a single type of macromolecule, nor can they be compared exactly between tissues and species.

A. CELLULOSE

The cellulose fraction consists of cellulose (which is often defined as a β-1, 4 glucan, but normally contains a small content of covalently linked mannose) plus small amounts of structurally distinct glucomannan and galactoglucomannan.

B. HEMICELLULOSE AND PECTINS

The hemicellulose fraction is largely composed of xylans, arabinoxylans and glucuronoxylans, with variable amounts of related polymers. The pectin fraction is extremely complex and contains rhamnogalacturonan, arabinogalactans and larger polymers containing all of these sugars with smaller contents of xylose, mannose, glucuronic acid, apiose (sometimes with KDO and aretic acid) and having a variable degree of

methyl esterification, acetylation and – Omethyl content.

Pectins are best considered as a family of modular polysaccharides in which the individual, major modules are homogalacturonan (or polygalacturonan PGA), rhamnogalaturonan (RGA which is a copolymer of galacturonic acid and torhamose) and rabinogalactan (AGa, which resembles the arabingalactan of cell walls). It is usually considered that the 'backbone' of pectins consists of regions of PGA and RGA, spliced end-to-end, with the AGa forming branches, attached to the RGA regions. However, there is evidence that some pectins, especially that of soy bean, can carry PGA attached to AGa, so producing a structure in which the AGa may be part of the 'backbone' and PGA may be largely present in side chains. These two structural models are not necessarily mutually exclusive: there could be a range of structural types possible with 'classical' pectins and soy bean pectins representing extreme forms. It should be noted that many (but not all) plant gums resemble pectins or parts of pectins and are likely to represent evolutionary variants of them, or the products of incomplete or unbalanced assembly.

In addition to the major 'modules' of pectins above, there are smaller branches within pectins, which include xyloglucuronan and rare sugars such as apiose, KDO (ketodeoxsyoctulosonic acid) and aceric acid, as well as small quantities of mannose and (unusually) glucose. The locations and roles of these sugars have largely become evident only recently and, in some cases, are still uncertain.

C. CELL WALL PROTEINS

The proteins of plant cell walls are a heterogeneous population. Many of them are both very highly glycosylated and of unusual amino-acid composition being particularly rich in glycine and hydroxyproline and sometimes low in their content of aromatic amino-acids. Several are rather insoluble in water and many are unusually resistant to proteolysis. They tend, therefore, to be associated, during extraction, with the 'cellulose' and 'lygnin' fractions, which are the least soluble fractions of cell walls. Several cell wall proteins are thought to be linear and triple-helical, but their organisation in the intact wall is largely unknown. Some cell wall proteins are probably carbohydrate-binding proteins, or lectins, which may be interacting with glycans of the cell membranes.

D. OVERALL STRUCTURE

Cellulose forms the major fibrillar component of the wall and is a large contributor to its tensile strength. Xylans are considered to be associated with the outside of cellulose fibrils and to cross-link them by forming a kind of three-dimensional 'chicken wire' structure. The pectins act as a kind of in-fill to this and are often visualised as a kind of hydrated 'plaster' to the cellulose and xylan 'laths', though their physical chemistry suggests a more complex behaviour than just this and they are clearly vital to the mutual adhesion of the walls of individual cells.

E. TYPES OF BONDING

Plant cell walls contain a wide assortment of chemical bonds, which vary greatly in their bond strengths and chemical stability. Covalent bonds are of many kinds, of which those that form the covalently linked backbones of the polysaccharides are the major contributors to the gross integrity and shape of most plant tissues, but those of proteins are also important. Chains of cellulose and xylan are relatively stable and do not show appreciable breakage either in dry specimens or under the conditions found in fluid-preserved specimens, even where the preservative fluid has become acidic over time, so long as its pH remains above 3.5 and no peroxidation occurs. Below that pH slow hydrolysis of glycosides is possible. Pectins and the arabinogalactans show more variation in stability among the glycosides that they contain. Arabinofurranoside linkages are labile and will tend to hydrolise appreciably below pH 5.0, over time and at ambient temperature. The rate of hydrolysis increases rapidly as the pH falls or the xylofuranosides, will behave in a similar way. In contrast, glycosides of non-esterified uronic acids are very stable and resist acid hydrolysis, so that the sugar ring may degrade or fragment in strong mineral acid before the glycoside breaks. Hence galacturonan chains are very unlikely to undergo hydrolysis even in old, acidified, preservative fluids and the same will apply to terminal glucuronic acid in xylans or callose. However, methyl esterfied galacturonans are liable to degrade by a different mechanism at any pH above about 8 to 8.5. This mechanism is that of a β-elimination reaction, in which the glycoside on the non-reducing terminal side of a methyl esterified residue of galacturonic acid breaks to form an unsaturation between carbons 4 and 5 of the esterified uronic acid.

Enzymatic catalysts of this type of reaction can occur and some fungi can produce pectin transeliminases.

Relatively weak covalent bonds occur between mineral and polysaccharide in cell walls. Boronate esters form bridges between apiosyl branches on polygalacturonic acid and are thought possibly to do the same with KDO and aceric acid. By analogy with other sugar boronates, they are likely to be reasonably stable at high pH, but to hydrolyse in mild acid and to be unstable and, possibly rearrange near neutrality. Silicate esters are likely to be even less stable and their study is extremely difficult, because of the ubiquity of silicates in water exposed to glass, or to plastic containing silicate filler, and the lack of any convenient radioisotope of silicon. Calcium ions can interact electrostatically with carboxyl groups of uronic acids, but can also coordinate with the hydroxyl groups of sugars and this may well be the more important interaction in the sequestration of calcium within plant cell walls.

It is unclear how far electrostatic interactions, for example between carboxyl groups of uronic acids and amino- or guanido-groups on proteins contribute to the stability of plant cell walls. In an aqueous environment, especially with salts present, such interactions may be limited and effective only over very short distances or in very specific local environments from which water is excluded or within which it is ordered. Exclusion of water, for example by lignification, will have a very large effect and will increase the effective bond strength of any electrostatic interaction: the dielectric constant of the environment may be massively altered.

Hydrogen bonds are extremely abundant in plant cell walls. Some form directly between sugars, as in cellulose, while others occur between glycan and protein, within proteins, or between glycan and/or protein and lignin. In some cases, there is a hydrogen-bonded interaction involving a molecule of water as a bridge. Such water is ‘structural water’ and is not readily exchangeable with bulk water, being removable only by severe, forced desiccation. Water itself is highly hydrogen-bonded and tends to form domains: this is a major consideration in thinking about how the removal or addition of water will affect the properties of stored plant tissues.

Hydrogen bonds have considerable potential for rearrangement, especially after the removal of the relatively tightly bound structural water and many will only very slowly revert to their former pattern on rehydration. This process, termed ‘curing’ makes the rehydration of severely dried specimens difficult and slow and lies at the bottom of problems of mechanical disruption that can occur if such rehydration is not performed slowly and very carefully.

4. CELL WALL FORMATION AND ASSEMBLY

After a plant cell has undergone mitosis, a new cell wall, flanked by new cell membranes, develops between the two daughter nuclei in the position of the equator of the spindle. It forms by the fusion of vesicles that move up between microtubules and, in its earliest form, it consists of a single layer of highly acidic material termed the cell plate. This is flanked, on either side, by cell membranes which are new plasmalemmata. Both the cell plate and the membranes extend at their edges by the accretion of new vesicles, the direction of the growth being determined by or reflected in the cytoskeleton. Eventually they fuse with the pre-existing wall and plasmalemma and the daughter cells become separated. There is evidence for the presence of acidic rhamnogalacturonan in the cell plate, but cellulose and most of the hemicellulose materials appear to be absent.

Before the cell plate has reached the pre-existing walls, a deposition of cell wall material begins at its centre, inward of the cell plate on each side, but external to the new cell membranes. This material is primary cell wall and contains pectins, hemicellulose and cellulose, in addition to glycoproteins and minerals. The primary cell wall, like the cell plate before it, grows outward to fuse with the walls of the parent cell. At this stage, the cell wall shows both elasticity and plasticity, being capable of extension and remodelling by the living tissue. It remains capable of structural and metabolic change and is dynamic. Its polysaccharides can undergo a variety of structural changes which can alter their physical properties and so enables the cell within, which controls this, to adapt and show differentiation of function. Locally, the membranes of adjacent cells may remain in contact, usually via desmosomes or hemi-desmosomes and at these points the wall is discontinuous and a distinctive polysaccharide, callose (a β-1, 3 glucan), is laid down around, but not across, the desmosome pits. It can also be deposited as a wound response. At this stage, a cell can undergo growth and extension and further cell division, or it may undergo secondary cell wall deposition, which is associated with its eventual differentiation and death.

Secondary cell wall deposition (or secondary thickening) involves the deposition of a new layer of cell wall internal to the primary wall. This new wall is generally much thicker than the primary wall and may be deposited in a spiral or reticulated pattern. It is rich in cellulose and contains some xylan, but the deposition of pectin eventually ceases. Cell wall plasticity is lost, but some elasticity remains, though the wall becomes more rigid. The remodelling of the wall draws to an end and finally the deposition of lignin begins, usually at the corners of the cells. This polymeric phenol forms by a free radical mechanism and may also be produced as a response to injury. It greatly reduces the permeability of the cell wall to water. In xylem, the cells die as their lateral walls lignify, while their end walls are degraded, so that vascular channels (xylem vessels) form. In phloem, sieve tubes form, with a little cytoplasm remaining within them in communication, via the plasmodesmata, with their companion cells. Thus, though the cells may be dead or moribund, their walls continue to serve functions in mechanical support and in providing the vascular systems of vascular plants.

5. PROPERTIES OF THE MOLECULESIN RELATION TO CELL WALLS

A. GENERAL

Cellulose is the major tensile element in plant cell walls. Cellulose fibres are longer than the individual molecules of cellulose and there are regions where the molecules overlap, which may be associated with their mannose content. The individual molecules are arranged in as antiparallel fashion, which maximises the opportunity for hydrogen bonding between them, so that they bind to each other rather than to water and form a strong, inextensible, non-slipping array. When water is further excluded by lignin, the effective strength of these hydrogen bonds is increased by about an order of magnitude so that wood is strong. In secondary wall the degree of polymerisation of the individual cellulose molecules is much greater than in the primary wall and the fibrils of cellulose are much more markedly aligned.

Xylan chains also tend to interact with each other and with cellulose by hydrogen bonds and they are conformationally similar to cellulose. However, xylans can contain appreciable amounts of arabinose, galactose, glucuronic acid and, rarely, other sugars and they show variability in this, for example, between angiosperms and gymnosperms, which can affect their properties by making their chains bend. In the *Rosaceae* the xylan chains are known to branch, but it is unclear how far this occurs in other orders.

The pectins are of particular importance in the maintenance of the integrity of plant tissues, because they are largely responsible for the adhesion *between* the cell walls, while cellulose and xylan are important for the cohesion *within*
the cell walls. In particular, the long acidic galacturonan segments of rhamnogalacturonans appear to be capable of strong association between adjacent molecules. Again, this is largely through hydrogen bonding between adjacent polyuronide sequences, though their pattern is somewhat different from those of cellulose, in that the usual configuration of the α-1, 4 galacturonan sequences in solution is not that which gives maximal hydrogen bonding, but, if stretched by about 20%, the chains can change conformation and maximise their inter-chain hydrogen bonding. The larger pectins that contain large branches of arabinose, galactose and other sugars appear to be less likely to associate, except where there are long, uninterrupted chains of non-esterified galacteronic acid, but they have a greater tendency to hydrate, with readily exchangeable water, at high relative humidity. Rhamnogalacturonans can show a different behaviour with water, in that they often take up ‘structural’ (ie almost non-exchangeable) water with high efficiency even at low relative humidities. The behaviour of pectins is further complicated by the sensitivity of their physical chemistry to their degree of methyl esterification and its pattern. What is clear, is that the distribution of water within plant cell walls will be highly sensitive to relative humidity, with the pectins probably accounting for a greater proportion of the water regain than their dry mass fraction within the wall might seem to imply and that they may well retain a very high proportion of the structural water within the wall even at low relative humidity (eg in dried herbarium specimens).

B. HYSTERETIC EFFECTS AND WATER REGAIN

If samples of plant tissues, their walls or the molecules that compose them are exhaustively dried, for example over calcium hydride, and are then allowed to equilibrate with atmospheres of known, defined relative humidity, at given temperatures, and are weighed, graphs of water regain against relative humidity can be plotted. Similarly, starting samples equilibrated with 100% relative humidity (ie saturation) can then be

equilibrated at lower relative humidities and similar graphs derived. For cellulose, the two curves thus obtained are close to each other and resemble similar curves for chitin and several other polysaccharides. The separation of the curves (hysteresis) is modest, but is still a little more pronounced than for most proteins. The water regain at high relative humidity is also modest, suggesting that exchangeable water is not a particularly marked feature of highly hydrated cellulose and that structural water is not present in large quantities. With the pectins, the picture is very different. Native homogalacturonan, especially if of low methyl ester content, shows marked hysteresis and a large water regain at high relative humidity. This implies that structural water is a notable feature of homogalacturonan, while both types of pectin, and especially those rich in arabinogalactan, can take up considerable amounts of exchangeable water. This tendency for water to associate with pectic polysaccharides is also reflected in their large 'partial specific volumes', which are measures of the amount of space occupied by a hydrated molecule when in aqueous solution. Carbohydrates in general show larger partial specific volumes than proteins and the pectins have some of the largest values of all. They also often show highly non-ideal behaviour in solution, for example in studies of their viscosity, which reflect interactions between and/or within molecules of the pectins which alter greatly with their dilution.

C. DEHYDRATION AND REHYDRATION

The long-term drying of plant material, as in herbarium specimens, leads to the 'curing' of cell wall polysaccharides (see above) and their becoming very insoluble and difficult to rehydrate. Elasticity is lost and the tissue becomes brittle. Rehydration is often possible if it is performed very slowly, through graded alcohol or glycerol solutions or by slowly raising the relative humidity around the specimen, but care must be taken to minimise differential swelling and attendant mechanical stress and to avoid the risk of bacterial or fungal growth as rehydration occurs. Thick specimens are particularly difficult to rehydrate, since the process will begin at the surface and may well proceed some way before water penetrates into the deeper layers, hence increasing the risk of mechanical failure. It should also be remembered that dehydration can similarly cause stresses and mechanical failure and that this may only be apparent when rehydration occurs in some specimens. Such failures are usually visible in larger dried specimens, especially in wood, particularly along natural cleavage planes such as medullary rays. Fracturing of this type in seasoned timber is termed 'shaking' and is a common feature of old timbers in buildings.

In general, pressed and dried herbarium specimens are sufficiently thin to rehydrate quite well, but it is seldom desired to rehydrate them. However, 'potted' specimens, kept under preservative fluids, may be much thicker and are likely to present problems if they leak and dry out. Mechanical failure may well have occurred before their condition is seen, especially if they are in closely packed, stored, reference collections that are only inspected occasionally and are kept – for good reason – in the dark. The restoration of such specimens can prove difficult and merely replacing the preservative fluid, without considering how to rehydrate with the minimal induction of stress, may be unwise and harmful. In some cases, where some damage has already occurred, it may be better either to dry the specimen completely or to infiltrate it with a different type of preservative. Even where a specimen has obviously fractured, the individual cells may remain well preserved and most of the cell-cell adhesion may remain except along the fracture planes. The proteins and nucleic acids of the tissue may be well preserved and some of the more robust enzyme activities may still be detectable. Such specimens should not be discarded without careful thought.

6. FIXATION AND FLUID PRESERVATION

Plant tissues are rather less likely to be subjected to fixation than soft animal tissues, partly because they retain their form relatively well on dehydration. More often, they are subjected to pseudo-fixation by dehydration in alcohol or alcohol-based fluids. However, some will have been fixed by true fixatives such as formalin or glutaraldehyde and the chemical mechanisms of action of these will not be materially different between plant and animal tissues. Proteins will have been cross-linked, primarily, but not exclusively, by way of amino- and guanido- groups of lysine and arginine respectively and most of these cross-links will be stable. A few will slowly reverse over time, if the specimen is not then kept in a preservative fluid containing a little aldehyde. As in animal tissues, true fixatives will cross link proteins so restricting molecular motions and leading to some degree of shrinkage. In plants, this is usually less marked than in animal tissues, because of the higher density of protein in most extracellular matrices of animals and the largely non-reactive structural 'skeleton' of carbohydrate in plant cell walls. Nevertheless, fixation may generate stresses in plant tissues, causing failure, and may impede attempts at rehydration in fixed specimens that have subsequently dried out.

In botanical collections, the most common preservative fluids tend to be alcohol-based, including alcohol itself, and fluids containing aldehydes, phenols or arsenites are unusual. Some plant specimens, if in fluids with a high water content, may tend to swell over time and their surfaces may become friable or may split. If alcohol is present, plant lipids may be leached out, especially if some of them are surface-active, such as saponins. Pigments, such as chlorophylls, phenolics and carotenoids, can also leach and the chlorophylls may lose magnesium and decolourise. If the fluid can oxidise and become acidic the risk of demetallising tetrapyrroles, such as chlorophylls, is increased and if transition metals, such as ferric iron, leach into the fluid or arise exogenously from mounts or caps of jars, for example, they may react with phenols in the tissue to produce dark staining.

7. ALGAL AND FUNGAL TISSUES

With the exception of algae of the *Characeae,* such as *Chara* and *Nitella* spp., algal walls have a different composition from those of the higher plants. Though they usually contain cellulose (a few contain chitin, below)they generally lack xylans and pectins, but possess other unrelated polysaccharides which, in some cases, are sulphated. They form a very diverse group, but share a common capacity for great hydration and, physiologically, normally exist at close to 100% saturation with water. On drying, some may 'cure' severely, but many algae can be dried and rehydrated very successfully. Heating, however, can lead some algal walls to a form of irreversible denaturation in which they show some shrinkage and become more leathery in texture. Overall, there is insufficient chemical information, except for those species of importance to the foodstuffs industry, and too much diversity in algal cell walls to generalise very far. This is a field in which more analysis would be highly desirable. The same is true of the higher fungi, where the analytical information on the composition and structure of their cell walls is seriously limited.

Fungal collections, for reasons largely of tradition, are usually held in the botanical sections of natural history collections. Like plants, the larger fungi have appearances which are largely determined or reflected by their cell walls, but the resemblance does not go far beyond this. Fungal walls have more in common, chemically, with animal cell surfaces than with plant cell walls and, even so, they are very much in a category of their own.

While some lower fungi (eg.chytrids and the *Hyphochitridiomycetes*) have cellulose in their walls, most often have chitin instead: this is a polymer of 2 deoxy, 2 acetamido-D-glucose (N-acetyglucosamine) which is largely analogous to cellulose. A few fungi appear to contain both, but the higher hyphal fungi have chitin. This forms a distinct layer in their walls adjacent to the cell membrane. Outside this is a thin layer of protein and, outside that, a much thicker layer of glucan and mannan, the latter being a highly elaborated form of the 'high-mannose' glycoprotein glycans found in all eukaryotes. In some yeast, such as *Saccharomyces cevevisiae* chitin is less abundant, a thick layer of glucan adjoins the membrane and a layer of mannan lies outside the glucan, but slightly interpenetrates it. In other pleomorphic yeasts, such as *Candida albicans*, hyphal and yeast forms are only marginally different from each other. Thus fungal walls differ with the growth form and species of the fungus and are very unlike plant cell walls. Many show other saccharides attached to the mannan and in some hyphal fungi the septa are chemically different from the lateral walls. Again, chemical information on the species found in natural history collections is seriously inadequate.

Fungal specimens are usually either dried or preserved in alcohol. Dried specimens generally show less shrinkage than in plant tissues, though a few, such as *Daldinia concentrica* are inclined to split along cleavage planes. The layers of the wall generally do not separate. Alcohol preservation generally works well, though extraction of pigment sometimes occurs.

Colouration and Fading: How do Pigments Become Degraded or Altered by Light and their Environment

- Bob Stoddart

A. Introduction

Many natural history specimens show distinctive colours, which often prove challenging to preserve over long periods and may be very labile to drying, fixation or exposure to preservative solutions or air. In most instances, the instability of colour is the unavoidable consequence of the chemistry necessary for a pigment to be coloured in the first place. Similarly, the tendency of pigments to leach is also a reflection of their necessary chemistry and its consequences for hydrophobicity or amphoteric behaviour. Some colouration in natural history specimens is, however, essentially physical in origin, its being essentially a product of reflectance, refraction, interference or some combination of these, but such colouration can also be unstable and the reasons for this are considered first.

B. Interference

Interference patterns can arise where light passes through or is reflected from a *regularly* patterned surface, where the spacing of the pattern is sufficiently fine to produce an effect in the visible part of the spectrum. Examples of such colours are known from the wing-cases of many beetles, the bodies of some flies, wings of various insects, scales of many fish and shells of some molluscs: they often show a silvered or lustrous appearance. Such colours originate in the same way as those produced by interference filters. Similar interference effects can also occur where light passes through transparent films of appropriate thinness and reflects from their upper and lower surface as in thin films of oil on water or the coatings of lenses in optical instruments or spectacles. In each case, rays of light are produced, either by transmission or reflection, the phases of which are sufficiently 'offset; to cause interference and so lead to the deletion of particular wavelengths from visible white light, so giving colour. Such colouration is extremely sensitive to changes in the periodicity or regularity of the 'interference filters' from which they arise. An alteration of period *with retention of regularity* leads to a shift in the wavelengths deleted and, hence, a change of colour. *Loss of regular periodicity* abolishes colour. This has clear implications for the possible effects of desiccation or fixation upon some biological tissues, where either type of effect above could follow. Where interference is dependant upon total internal reflection at a boundary where there is a change of refractive index, it may also be very sensitive to any alteration in refractive index, such as that produced by immersing something normally viewed in air in a preservative fluid, or its converse – such as taking a fish out of water. The effect may be either the loss of colour or its being visible only from a different viewpoint.

C. Reflection and Refraction

Reflection and lensing effects occur on occasion. Many vertebrates show reflection from layers behind the retina when viewed 'head-on': typically they are species which hunt nocturnally or in poor light, such as cats, and the reflection affords a double-pass of light to the sensors in the retina. A few plants, such as the protonema of the moss *Schistostega*, have lens-like cell walls that focus light onto the chloroplasts, from which it is reflected, producing a shimmering gold appearance. Any change in the curvature of the lens or in the refractive index of the medium outside disturbs the optics, abolishes the effect and colour is lost. Hence, such apparent colour is extremely labile to drying or immersion in preservative fluids.

D. Pigmentation

Most colouration of natural history specimens arises from pigmentation. A few organisms may fluoresce if excited at visible wavelengths and many more are fluorescent under ultra-violet light but, in general, colour arises from the absorption of particular wavelengths from transmitted or reflected white light, so that the unabsorbed wavelengths give rise to the observed colour. Bioluminescence is a property of living cells and is not a phenomenon seen in dead tissues, except where it is the result of putrefaction and arises from the processes of decay – generally from a saprophyte.

When pigments absorb light they do so by taking up photons and promoting electrons from lower to higher energy levels: thus the pigment absorbs energy from light and stores it in the form of more energetic electrons than before. This energy can be dissipated by allowing the electrons to fall back to lower energy levels, usually in a series of steps, with the re-emission of energy in the form of photons of lower energy than those which caused the initial excitation. This is fluorescence. Alternatively, the energy can be dissipated in chemical reactions which will alter the pigment, or it can be transferred to other molecules which may, in

turn, fluoresce or undergo chemical change. It may be lost in the activation of water molecules and can eventually appear as heat by a variety of routes. However the energy is lost, the pigment cannot absorb again until its electrons have returned to a lower energy level.

The energy levels of the electrons in a molecule or atom are not continuously variable, but have precise, fixed values and transitions between them *must* occur by the absorption or loss of specific amounts of energy. Hence a pigment will absorb at specific wavelengths that correspond to the energies of these transitions. Most pigments in natural history specimens are organic chemicals; a few, such as haem or chlorophyll are organometallic compounds in which a metal (often a transition metal such as iron) interacts with an organic molecule. Pigmentation by metals alone is rare.

The energy levels of the electrons in a molecule reflect its chemistry. In most naturally occurring pigments the part of the molecule that absorbs visible light (the chromophore) is a structure extending over several atoms, such that some electrons can move across the domains of several atomic nuclei (ie. They occupy *molecular* orbitals, rather than being confined to *atomic* orbitals). The length of the system is the primary determinant of the wavelengths absorbed, but this is modified by the nature of and polarity of interacting substituent groups which may 'fine tune' the colour produced. Commonly pigments show extended chains of linkages which are usually represented as alternating single and double bonds between carbon atoms. The carotenoids (fig 1a) are a good example. As a group of pigments they share a common backbone of isoprene units in which the chromophore is composed of alternating double and single bonds and confers the ability to absorb with maxima at about 485, 455 and 425 nm, so generally giving a yellow, orange or red pigment. The exact shade of the colour is modified by variable, linked structures at each end of the chromophore.

Structure of this type will show all the reactions expected of this class of molecule (ie. all the reactions of olefinic structures). Any such reaction which interrupts the alternating sequence of conjugated double bonds in the chromophore will alter or abolish colouration. If the interruption is near the end of the chromophore the colour may not be wholly lost, but absorption will change to a shorter wavelength and so the colour will alter. Interruption near the centre of the chromophore will shift absorption out of the visible range of wavelengths and colour will be lost. If an addition occurs which extends the chromophore, colour will also change, as the wavelength absorbed will be longer, provided that it remains in the visible range.

Carotenoids are susceptible to reducing agents, which convert – CH = CH – to - CH2 – CH2 - and so interrupt the chromophore. Oxidising agents which form epoxides with the double bonds can split the chromophore and so also cause loss and change of colour. Many types of addition reaction are possible, such as halogenation by chlorine or bromine, so that solutions of hypochlorite can cause bleaching. Sensitivity to oxidation – reduction state should particularly be considered when considering pigments that are chemically related to redox carriers such as the pteridines and carotenoids. Reduced or oxidised, colourless derivatives of pigments may remain even when a specimen has apparently faded.

Some pigments, such as the porphyrins (fig 1b), have chromophore systems which are conjugated in such a way that they form large loops. In systems of this kind, the loss of one double bond does not interrupt the chromophore completely and, while colour may be modestly altered, the molecule as a whole will still absorb light. If the large ring is broken, however, the molecule will become a more or less linear tetrapyrrole (as in the conversion of haem to bile pigments), the length of the chromophore will be increased and the colour of the pigment will alter.

A further change will also occur. Chromophores absorb light most efficiently when light waves are aligned with the axis of the chromophore and least efficiently when they are at right angles to it. Thus a linear chromophore absorbs effectively only when in a particular orientation. In contrast, a molecule such as haem can absorb effectively in a much wider range of orientations and, because a solution of a pigment will have molecules in all orientations within it, a solution of (for example) a porphyrin will have a larger molar extinction coefficient than (for example) will a carotenoid. Hence the conversion of haem to a bile pigment will lead to less efficient absorption of light.

Where tetrapyrrole structures, such as porphyrins, are conjugated to metals, as in haem or chlorophyll, some of their absorption of light is attributable to their interaction with the metal. If the metal is lost, colour will change, as in the chlorophylls. Occasionally, the presence of metal shifts a very intense absorption band from the near ultra-violet into the visible range, generating very deep colouration, as in the manganese-

porphyrins of some avian feathers. If the metal is removed by weakly alkaline solutions, massive loss of colour occurs.

The effects of polar substituents upon the colour of pigments is well illustrated by the anthocyanin pigments of red, purple and blue flowers (fig 2) in which the particular shade of colour is highly sensitive to the number and position of – OH and - O methyl substituents upon the core structure and to the nature of the sugar to which the anthocyanidin is glycosidically linked. Hydrolysis of the glycoside affects both the colour of the pigment and its water – solubility. The anthocyanidins are also sensitive to pH and their colour depends upon whether they are present in charged, uncharged or quinonoid forms. Litmus is a mixture of anthcyanidins and the colour change is exploited in indicator paper. These changes are reversible, but have implications for changes in preservative fluids and atmospheric acidity.

The phenolic hydroxyl groups of anthocyanidins, in common with other phenols, can form adducts with ferric ions to yield deeply coloured coordinaton complexes. This change can occur in old specimens in fluid collections over time and may be followed by oxidative damage catalysed by the metal ions.

Many plant phenols show a tendency, over time, to undergo oxidative polymerisation, with attendant formation of larger chromophores and deeper and more red-brown or blackish colour. This process occurs naturally during lignification and on wounding of many plant tissues and is catalysed by polyphenol oxidases which can be relatively resistant to denaturation and may persist in preserved specimens for an extended period. Their action is the basis of tea fermentation, in which polymeric derivatives of catechins, gallocatechins, epigallocatechins and epigallocatechin gallates are oxidised; the result is the range of colours of tea, especially of black tea.

The chemistry of many classes of pigment also confers upon them characteristic patterns of solubility and susceptibility to leaching in specimens stored in preservative fluids. The linear, rather hydrophobic carotenoids and the bile pigments are sparingly soluble in water, but more soluble in alcohol and are readily absorbed into fat. Porphyrins, the chlorophylls and related pigments tend to be soluble in polar organic solvents, especially in the presence of a little acid or alkali. Anthocyanins and the smaller plant phenols are rather soluble in water and, in some cases, in aqueous alcohol, but the larger phenols are insoluble in most solvents. All of this and the pH-sensitivity of some pigments has obvious major implications for the design of preservative fluids.

No form of preservative is wholly suitable for all pigments and some preservatives and fixatives may interact with them chemically. Picric acid can interact with DNA by its insertion between the bases of the double helix to form a charge-transfer complex and it can, at least in principal, show similar behaviour with pigments that have flat ring structures, especially if they contain basic groups. This can produce large shifts of colour. Formalin is a reducing agent and, over time and in the presence of oxygen, it can alter colours. Alcohol, acetone, chloroform and may other solvents can leach some pigments. However, the 'signatures' of the pigments are not wholly lost and their colourless derivatives may remain in the specimen, or the fluid around it, and be capable of identification by modern microanalytical methods. This should be considered when assessing whether specimens have deteriorated beyond use or salvage.

Digestion of Tissues by Invading Fungi: Questions and mechanisms

- Bob Stoddart

Microfungi are ubiquitous in air and soil, except in the most extreme of conditions. Most are saprophytes or harmless symbionts: very few of the total are pathogenic. The principal reasons for the variety of fungal pathogens, either in animals or plants, are twofold. First, many fungi have such exacting requirements for growth that they cannot proliferate at body temperatures or obtain all their nutritional requirements as pathogens. Second, *living* organisms have a variety of protective mechanisms, some passive and others active, which are very efficient in defence against fungi.

When a tissue dies, its passive anti-fungal defences may still function to some degree, but its active protective mechanisms soon cease to operate and so dead tissues are potentially vulnerable to fungal attack. In natural history collections attempts are made to afford compensation for the loss of active defence by using exogenous, additional protection through fixation, drying and preservatives such as are used in fluid collections and in tanned specimens. In general, such protection is reasonably effective, but specimens do, periodically, show evidence for the presence of fungus and this raises several questions as to the source of the fungus, whether it is alive, what it has done to the specimen – or will do to it, the source of the fungus and whether other specimens are at risk. There are, occasionally, considerations of human risk also to be made. The central question, however, is whether the fungus has been acquired after the death of the tissue, or was present in life, before the specimen was prepared.

Fungi are pleomorphic and their appearance can be greatly affected by the site and conditions of their growth. In general, yeasts are not invasive in their yeast forms, but tend to grow in suspension or on surfaces. They are likely to affect the surface of a specimen and to spread only locally, unless they are able to survive in preservative fluids or spread across cavities. They may disperse in dusts, as may fungal spores, which often resemble yeasts when seen histologically. Hyphal fungi are potentially invasive of tissues, though they do not necessarily invade and may simply produce surface growth which may be dense. They may spread easily through fluids and extend across cavities, but do not always do so. If their growth conditions become adverse, for example by drying, they may die or form spores and the mechanisms of spore formation are variable between species with some being able to form arthrospores simply by the fragmentation of hyphae. As a general rule, if the fungal growth on a specimen, when examined microscopically, shows evidence of hyphae or pseudohyphae invasive growth should be presumed as a starting point, even if no hyphal invasion can be seen. If any sporangia or possible arthrospores are visible, there should be a presumption that they have dispersed into the immediate environment of the specimen and may be present on nearby surfaces or in surrounding fluids. If clear-cut sporangia are seen, it is sometimes possible to identify the species of fungus concerned, or, at least, to place it within its taxonomic group and this may aid in predicting the types of damage that it may have done.

If fungal infection of a specimen is suspected, a sample should, if possible, be taken for microscopic and histological examination to determine, if possible, whether invasive fungus is present. In this, care should be taken to distinguish between fungal hyphae and features such as blood capillaries or small vascular channels in plants, which can imitate dead or dying hyphae. If fungus is confirmed, a series of questions follows.

a. Was fungus present in the living tissue?
Animal tissues generally respond to fungal invasion by a variety of cellular mechanisms. In mammals these include responses by neutrophil polymorphs, lymphocytes and macrophages, which lead to collections of some or all of these cells around fungi and the engulfment of fungus by macrophages. In lower animals there are functionally equivalent cellular responses, though the separate cell type may not be identifiable. However, the presence of fungus inside phagocytic cells is a clear indication of its presence before tissue death and implies that it was part of the specimen *ab initio*. In plants, the tissue responses are different, but the presence of polyphenols (which may be blue or brown) around the fungus is an indication that the fungus was present before tissue death, or soon after.

In mammals and several other vertebrates, fungi can induce the generation of immunoglobulins directed against them and this can lead to the precipitation of amorphous immune complexes, which stain pink with

haematoxylin and eosin, on the fungal surfaces. Such precipitates (the Hoeppli-Splendore phenomenon) occur only in living tissues and also provide evidence for a fungal presence in life.

b. *Is the fungus still alive?*
This may be very hard to determine. Sampling by an experienced microbiologist and subsequent culture may provide an answer, but care is needed to ensure that the fungus is not a recent exogenous contaminant. Careful morphological comparison with that in the tissue may be required, but the pleomorphism of many fungi is a difficulty.

Many fungal hyphae appear to show considerable lengths of dead material even under condition favourable for growth and demonstration of metabolic activity can be problematic *in situ*.

c. What sort of fungus is it?
Culture may provide the answer to this, given the caveat in 'b)' above. Histology may also help.

Aspergillus spp. Generally show quite narrow hyphae of reasonably uniform diameter, with frequent and complete septa. Where growth is confined, dilated 'flask' cells may be seen. Branching is usually dichotomous and even, with adjacent hyphae often branching in synchrony to produce 'fairy ring' effects. If sporangia are seen (eg in cavities or at surfaces) they resemble holy water sprinklers (aspergilli) and their shape, size and number of ranks of spores (sterigmata) may indicate the species. *Penicillium spp*. Are closely related to *Aspergillus* and their hyphae are quite similar, but their sporangia are simpler.

Fusarium spp. Also have narrow hyphae, but few septa and do not branch so often or so regularly.

The Zygomycetes, which in Britain are chiefly represented by species of *Mucor* and *Absidia*, are common and rather aggressive fungi. Their hyphae are often broader than those of the species above, less even in diameter, branch rather irregularly (and often at right angles) and tend to look rather vacuoled or empty. They have a bluish stain with haematoxylin and eosin and may show zygospores. In specimens from the USA species of *Rhizopus* may be found, which show even more irregular and broad hyphae. Zygomycetes are commonly found on waste foodstuffs. A wide range of rusts and other common plant pathogens my also be encountered as may dermatophytes (the organisms of ringworm and athletes foot). These last usually fluoresce under ultra-violet light and the colour of the flourescence may indicate the species.

Stuffed mammals and skins may grow organisms such as *Trichosporon capitatum,* which causes piedra in humans. These invade hair shafts, causing the hair to become brittle and break. Organisms of this type are known also to grow on house dust and rotting wood.

A wide range of organisms grow on timber and in soil. They may occur, for example, in archaeological bone and are abundant in earth from graveyards and cemeteries. Several of them are inherently coloured (generally brown). They may grow as masses of parallel hyphae ('rhizoids') and show mating between hyphae, indicated by the presence of crescent shaped unions (termed 'clamps').

In general, it is difficult to identify fungal species without culture, but recognition of the main taxonomic groups is often easier and may indicate the source of the fungus.

3. Where does the fungus orginate?
Though fungi are effectively, everywhere, particular species are associated with particular environments and with certain sources of specimens, so that it can be possible to identify likely sources of infection and to respond accordingly.

Museums are often in or near old buildings, in which there may be decaying wooden window frames, roof timbers – and sometimes floor timbers – affected by dry or wet rot and lawns (and grass clippings) may be nearby. All of these may harbour fungi, as may the dust between floorboards or underneath floor coverings. Visitors may introduce dermatophytes and the waste from cafes in or near the museum may harbour zygomycetes. In country towns, dusts from cornfields or from hay may carry large burdens of fungal spores over considerable distances. In general, this does not seem to matter as much as might be supposed, provided that the fungi and their spores are not disturbed too much, but building operations, such as re-roofing, re-flooring, demolishing walls or replacing decayed window frames *are* a real hazard, as they are for leukaemic patients in hospitals. Even building operations some hundreds of yards from museums pose a risk.

Air conditioning systems afford a further problem in that they may disperse fungal infections from specimen to specimen, especially if they are not adequately filtered. The position of their air intakes relative to dustbins, garden waste and building work should be considered. In particular, thought should be given as to whether the outfall from venting a hazard, such as a kitchen, waste store or even – in the author's experience - a mortuary lies too close to, or upwind of, the intakes of an air-conditioning plant.

4. What has the fungus done to the tissue or what may it do?
Where fungi invade tissues, they generally do so along natural routes of access and cleavage planes. In animal tissues they tend, even in life, to invade airways (especially in birds, reptiles and some insects) and they can spread rapidly through air-filled cavities such as lungs, the stomata of leaves or the empty marrow cavities of archaeological bone. They will also track through vascular systems in plants and, sometimes, in animals and commonly enter bone by this means. In general, fungi do not invade cartilage at all easily and they are slow to penetrate the matrix of cancellous bone. Indeed, it is often striking that dense mats of fungal rhizoids may be within the trabecular bone of marrow cavities while sparing the hard cancellous bone. Zygomycetes are notably more able to penetrate soft tissues, whatever their texture, than are most other fungi.

The nature of the damage done depends on the enzymes produced by the fungus and this is often variable, even within strains of a given species. In some forms of *Aspergillus spp*., *Mucor spp*. and in a wide range of plant pathogens, there is production of pectolytic enzymes. These are, variously, pectin methyl esterases, pectin transeliminases and polygalacturonases of several types, which together degrade simpler pectins, including those of the middle lamella and cause individual plant cell walls to separate from each other. This process is termed 'maceration' and can leave the cells themselves reasonably intact. In contrast, celluloses and some xylanases will damage the entire wall and make it swell or fragment, often with injury to the cells within. Proteolytic enzymes are common fungal products and tend to be of broad specificity, so that they can degrade a wide range of proteins, especially in animal tissues. Fungi can also produce a wide range of lipases and nucleases, but most species only produce a limited range of lytic enzymes. For this reason – and because of the inhibiting effects of fixatives and preservatives – only a limited range of fungi are likley to cause major damage to natural history specimens and most of these are Zygomycetes, Aspergilli and related groups or fungi such as dry rot (*Merulius lacrymans*).

5. Is the fungus hazardous to humans?
Apart from their tendency to induce allergy in susceptible individuals, most of the fungi encountered as problems in collections are unlikely to cause serious hazards for museum staffs or the public, but there are some exceptions. Anyone with an immune deficit or a known hypersensitivity to a fungus should avoid contact with them and, especially, avoid inhaling spores. Care should be taken in handling any fungus growing on an animal tissue until the species or major taxonomic group is determined and culture should be attempted only by persons with appropriate microbiological training and is a suitable laboratory, though this is as much for the safety of the collections as of the staff.

Some few specific fungal species present a high level of hazard in general and they should be borne in mind, even though the chances of their being encountered in Britain are *very* low. *Coccidioides immitis* could possibly be present in specimens of desert-swelling mammals from the South-West USA and a few parts of West Africa. On no account should any attempt be made at culture, even if its presence is suspected, without the use of high-grade containment facilities. Other potentially dangerous species that might be encountered include *Blastomyces dermatitidis* (on animal tissues and decayed wood, especially from the area around Tennessee, USA), *Paracoccidioides brasiliensis* (from similar sources in the south of the USA, Central America and some areas of South America) and *Sporothrix schenkii* (on old softwood from various sources). If museum roofs are being inspected, areas fouled with old pigeon droppings should be avoided or protective clothing should be used in order to avoid inhaling *Cryptococcus neoformans*, which can be a dangerous pathogen and has an almost world-wide distribution. Finally, any decaying specimen, especially a dried-out pot, that smells of garlic when opened should be handled with care and in a fume cupboard. It may have been exposed to an arsenical preservative, such as sodium arsenite or arsenious oxide and be releasing arsine (arsenic trihydride), a highly poisonous gas, as it putrefies, whether by fungal or bacterial action.

The Thermo-lignum Pest Control Treatment

- A.M.Doyle The Natural History Museum, Palaeontology Conservation Unit

Abstract
The commercially available Thermo Lignum© process of pest control uses high temperatures combined with controlled relative humidity to provide a safe and practical option for large-scale pest control treatments. It has previously been tested on a range of collection material from the Natural History Museum. Preliminary investigations have now been carried out with respect to possible effects on D.N.A. on selected entomological specimens. These have shown that, within the test parameters, there is no known detrimental effect on these specimens as a result of this procedure. Further research is required on a wider range of material to establish the potential scope for the treatment for Natural History collections.

1. Introduction

Thermo Lignum© is a pest eradication process that controls relative humidity within a large capacity chamber throughout a heating-cooling cycle that includes three hours at a maximum core temperature of approximately 52°C, guaranteeing 100% pest mortality.

Previously published research (Ackery P.R., Pinniger, D Doyle, A., and Roux, K 2005)**,** indicates that the Thermo Lignum© process can provide a suitable and safe pest-control method in a variety of museum standard, lidded entomological drawers. By applying certain protocols and controls to transportation, packaging, placement and spacing of drawers within the chamber as well as appropriate chamber environmental levels, the treatment did not create extreme humidity changes which could potentially damage specimens and storage materials.

The next phase of the research was to identify whether this technique would have detrimental effects to D.N.A. to prevent or interfere with current and future molecular research as well as other physical or chemical effects. A research paper was published from which this article is derived (Ackery. P, Testa. J.M., Ready.P.D. Doyle.A.M., Pinniger. D.P. 2004).

2. Procedure

Selected entomological test specimens comprised;recently caught noctuid moths, 10-year old sphingid moths and 20-year old nymphalid butterflies (Danainae. All of these had a known history and have remained untreated by historical or contemporary pest control techniques (i.e. fumigants etc). (See table 1) This enabled us to test empirically whether D.N.A. could be extracted and amplified from the treated specimens and to establish that the resultant sequences were lepidopteran DNA.

2.1 The treatment cycle

Undergoing established Thermo Lignum© processes, the selected entomological specimens were subjected to three hours at 52°C. Imposed controls on relative humidity reflected the requirements of other non-entomological items included in the test run (Thermo lignum© is a commercial business and has client's time booked), peaking at 57% Rh compared to an average collections storage level of 47% Rh around the period of the test run (12.6.2002).

2.2 Specimens from which DNA was amplified

D.N.A. fragments of the correct size-range (300-400 base pairs) were successfully amplified by polymerised chain reaction (P.C.R.) from most samples although this was not as successful for the control samples of *Danaus affinis* as it was for the test samples of the same species or the control and test samples of the other two species. 4/8 of the *Danaus* controls amplified, but the smaller quantities of PCR products obtained from them indicated that less amplifiable DNA had been successfully extracted than from all the other samples.

An interesting *Danaus* result showed that this particular Thermo Lignum© operational cycle might have actually enhanced the efficiency of DNA extraction from old specimens, probably by some form of rehydration. (Ackery. P, Testa. J.M., Ready.P.D. Doyle.A.M., Pinniger. D.P. 2004). This result needs to be re-examined and re-tested to confirm that this is repeatable and is as a direct consequence of the Thermo-lignum© process. (It was however confirmed that there were no contaminants of human or other origins).

3.0 Other ancillary effects upon materials; storage and packaging materials and other specimens
In 1999, with the co-operation of Thermo Lignum©, a wide selection of natural history specimens and associated collections management products such as packaging materials, adhesives, resins and inks were tested along with controls. As was expected, there was no apparent detrimental affect to the packaging materials and inks and there does not appear to be any detriment effect on any of the adhesives (the melting point of these adhesives is higher than the temperature of the Thermo Lignum© chamber although adhesives are subject to chemical change over time).

The only visibly noticed effect was of greasy marks on specimens of oily fish. However, similar problems which were anticipated with some moth groups that have high grease content, particularly castniids, cossids and noctuids, did not occur. Although some verdigris clearly became detached in transit which shows that transportation issues need to be addressed if a treatment 'run' does not happen 'on site'.

(Verdigris forms on brass entomological pins when copper in the brass pin reacts with body fats and acids resulting in the characteristic and all to familiar greenish greasy deposit).

4. Conclusions
From the results of these basic investigations, the aim is to extend these preliminary results with different batches of the same species and other insects from different orders. Those specimens which were used in this study will need re-analyzing in the near future to investigate any longer- term detrimental effects of the Thermo Lignum© treatment as well as identifying any other form of investigative process that may be suitably employed to detect any form of damage. In addition, the suggestion of a possible relationship between re-hydration and enhanced efficiency of DNA extraction needs to be investigated further as does further work on inherently 'greasy' specimens and verdigris.

Finally, as understanding of historical conservation techniques become better understood and researched; specifically, previous treatments with chemicals, Thermo lignum© should be investigated as a possible method for removing unwanted and hazardous pesticides in a safe and controlled manner.

5. Acknowledgments

Sincere thanks to Karen Roux, Paul Leary and Werner von Rotberg of Thermo Lignum© for their co-operation and interest throughout our collaboration and all of my colleagues at the Natural History Museum who worked on this project.

6. References

1. Ackery, P.R., Pinniger, D Doyle, A., and Roux, K.. Heat treatment of entomological drawers using the thermo lignum heat process. Collections Forum, 19 (1-2) (2005), pp 15-22

2. Ackery. P, Testa. J.M., Ready.P.D. Doyle.A.M., Pinniger. D.P. 2004, effects of high temperature pest eradication on DNA in entomological collections. *Studies in Conservation* 49 (2004) pp 35-40

Table 1. Lepidoptera specimens analysed

Species	**Specimen age**	**Comments**
Danaus affinis	20 years old	Pinned specimens from the collection
Clanis bilineata	10 years old	Dried and stored in packets
Agrotis exclamationis	August 2002	Collected from the Museum Wildlife garden

NEWS

Notices, Adverts & Meetings

Meetings:

SPNHC 22nd Annual Meeting 2007
Building for the Future: Museums of the 21st Century
Meeting Dates: May 21-26, 2007

Registration: Early Bird Registration is now over. If you have not registered yet, we encourage you to do so soon. See the conference web site for information.

Field Trips: Collections tours are approaching maximum numbers. Sign up soon to guarantee your spot – or, register instead for a unique Minnesota experience: hiking through restoration prairie, enjoying unique geological forms formations in an urban setting, birding along the Mississippi River from St. Paul to Historic Red Wing (yes, where the boots come from) or relaxing canoe trip along the scenic St. Croix River. May in Minnesota promises to be glorious!

Committee Meetings: The schedule for Committee Meetings (Monday, May 21) will be posted on the web site in April, via NHCOLL, and in an email to the membership.

Workshop: The workshop is filling up fast! The workshop will run for one and a half days. It starts with a full day at the Minnesota Historical Society. The second day will be held at the Science Museum of Minnesota. You will have the opportunity to compare solutions. Benefit from our successes and see what each would have done differently.

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This will be a two-step process, consisting first of a call for paper and (extended) poster abstracts and second, a call for full papers and updated, short poster abstracts. In order to adhere to the planned selection and editorial process, authors, coordinators, peer review committee and editorial committee members are strongly advised to take into account the "Schedule for Preprint Production" that is available at the ICOM-CC website.

http://icom-cc.icom.museum/TriennialMeetings