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Author(s): Norris, C A.

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THE "BONES" MEETING - Monday, 20th February, 1995 at the Grosvenor Museum, Chester.

Editors Note:

53 people attended the meeting.

The morning session was chaired by Maggie Reilly, Hunterian Museum, Glasgow, and talks were given by James Rackham (an Environmental Archaeologist), Chris Norris, Kate Andrew (Geological Conservator and Collection Care Consultant), and Paul Finnegan (Natural History Centre, Liverpool Museum).

The afternoon session was chaired by Steve Garland, Bolton Museum, and talks and demonstrations were given by Kate Andrew (again), Clem Fisher, Geoff Yates, and Rosina Down (University College London).

Three papers based on the talks are published here; it is intended that papers by James Rackham, Kate Andrew and Rosina Down will appear in the next issue.

THE USE OF OSTEOLOGICAL COLLECTIONS FOR SYSTEMATIC RESEARCH

Dr Christopher A. Norris, Zoological Collections, The University Museum, Parks Road, Oxford OX1 3PW

Introduction

Osteological material has a very great significance in systematic studies of vertebrates. As Szalay (1994) states, its use ensures the vital continuity between living and extinct forms. Even with the great advances in molecular techniques made over the last twenty five years, the osteological collections of the world's museums remain in constant demand as a source of taxonomic data.

This paper briefly reviews the categories of research methodology that can be employed when using osteological materials for taxonomic purposes and their applicability to the range of osteological collections available in museums. The types of bone most commonly used are described and, in conclusion, some of the problems and opportunities for managers of osteological collections are discussed. The paper concentrates to a large extent on mammalian systematics, but the general principles are applicable to most types of vertebrate material.

Research Methodologies

Broadly speaking, the systematic research methodologies employed on bones can be characterised as "direct" or "indirect." Direct methodologies involve the use of the actual bones as a source of data, be it in a quantitative or qualitative form. In contrast, indirect methodologies use the bone as the starting point for the analysis, but derive their final result from the molecules contained within the bone; for example, through the comparison of homologous sequences of DNA.

Direct methodologies

1) Quantitative studies. These involve the measurement of the specimen (using a variety of dimensions) and the replication of these measurements across a large number of other specimens. Analysis of the resulting data using a specialist software package produces phylogenies based on numerical similarity. The strength of such methodologies lies in their ability to distinguish the subtle differences in proportion that may separate populations of a species, or species within a genus. However, this same sensitivity makes such methodologies unsuitable for studies of more distantly related taxa, where the magnitude of the differences may swamp the analysis.

There are a large number of confounding variables in such analyses, whose elimination tends to dictate the requirements in terms of material. A largen umber of specimens is required, in order to reduce the effects of individual variability (e.g. in size). It is helpful to have access to series of specimens from the same locality, in order to separate within-locality variation from between locality variation. Wherever possible, specimens should be compared with those of the same age and sex, to reduce the effects of variation based on these factors (e.g. sex-based dimorphisms). It is also important to have a set of measurements that may be accurately replicated. The type of collection available may have a marked effect on this. For example, in the taxonomic review of the marsupial genus Phalanger carried out by Menzies & Pernetta (1986) a large proportion of the specimens used were hunting trophies obtained from indigenous peoples in New Guinea. In such specimens the cranium had usually been shattered to allow removal of the brain. The specimens were thus reduced to the orbito-rostral and palatal areas of the skull (see below). Although more complete specimens were available in the museum collections utilised by Menzies & Pernetta, the need to ensure replicability across all the samples meant that the study was restricted to a set of palatal dimensions (figure 1) which represented the "lowest common denominator" of the material available.

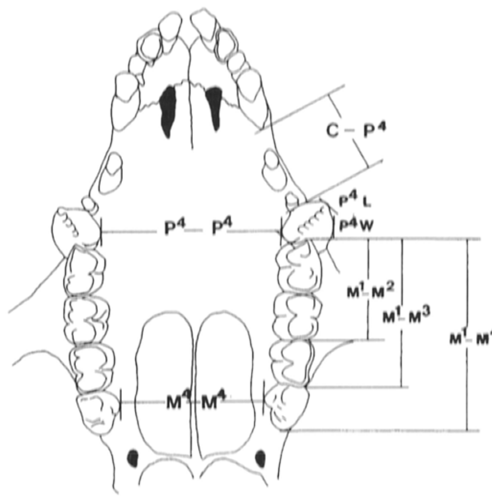

Figure 1. Palatal view of the skull of a cuscus (Phalanger), showing the dimensions recorded by Menzies & Pernetta (1986).

The demands of quantitative studies, in terms of the size and characteristics of the collections required and the quality of the associated data, are such that they cannot be effectively undertaken in any but the largest of collections.

2) Qualitative studies. Such studies involve examination and categorisation of a variety of distinctive morphological features of the specimen. An example would be the relationship between two bones in the skull - do they meet directly at a suture, is there a third bone separating them, etc? Comparison of a number of specimens within the same taxon enables a judgement to be made as to whether the formation of the character is consistent for that grouping. If it is, then it can be added to a set of characters to be compared between taxa. The observations are converted into a binary format for each character (e.g. 1 = bones touch; O =bones are separated) which can be entered into a specialist software package which compares the data sets and produces a phylogenetic tree based either on overall similarity (phenetic) or specific character distribution (cladistic). Such studies are best employed for elucidating the relationships between higher level taxa (i.e. at a supra-generic level). At lower levels the analyses are handicapped, because the differences between taxa at the specific and subspecific/population levels are often not of such a magnitude that they can be picked out by eye.

Overall, the effect of confounding variables in qualitative studies is much smaller than for quantitative work. For this reason, qualitative studies represent a less demanding discipline in terms of the quality of material available. In contrast to quantitative studies, species coverage in "breadth" rather than "depth" is required. This makes qualitative work suitable for moderately large collections such as those at Oxford, where the emphasis on the teaching role of the Zoological Collections has led to a wide species coverage, but only limited numbers of specimen per species.

Indirect methodologies

Over the past five years, much interest has been generated regarding the potential for extracting molecular information from museum specimens. In particular, the development of the polymerase chain reaction (PCR) in the late 1980s has made it possible to amplify selected sequences of DNA from the quite small fragments available in preserved materials, to the point where they can be sequenced. This interest has been heightened by a number of high- profile successes, notably the extraction of "ancient" DNA from a skin of the extinct marsupial "wolf" *Thylacinus* (Thomas et al, 1989), a 13,000 year old giant ground sloth of the genus *Mylodon* (Paabo, 1989) and a 17-20 million year old fossil *Magnolia* leaf (Golenberg *et al*, 1990). Thomas *et al* (1990) have carried out a study of the relationships between populations of the rodent *Dipodomys panamintinus* on the California Channel Islands based entirely on DNA extracted from museum specimens (see also Diamond, 1990). The use of museum collections for such work may increase in the near future, but a number of caveats should be attached to this statement. Firstly, such studies remain the preserve of the well-resourced and highly-specialised molecular biologist and are currently beyond the means of all but the largest of museums. The degraded nature of ancient DNA makes extraction particularly problematic, with Paabo (1989) reporting that the condition of DNA from a specimen of 100-200years' age is little better than that of a specimen of many thousands of years' age. Consequently, the expertise required is restricted to a small number of groups worldwide. Although DNA can be extracted from bone, osteological material is not ideal: tissues with a high cell-count, such as muscle, kidney, testis or ovary, tend to be more suited to such work. Consequently, extractions from bone are only really worthwhile when this is the only material available. For systematic studies, this is only likely to be true in the case of extinct or extremely rare specimens. In such cases, the collections manager is faced with some difficult ethical decisions, which are discussed at length below.

Classes of Bone used for Systematic Studies

The Postcranial Skeleton. The postcranial skeleton is made up of two of the three skeletal systems that make up the vertebrate body; the axial (i.e., vertebrae, ribs, sternum and, if present, gastralia) and appendicular (i.e., limbs, girdles) skeletons. Whilst there is potential for qualitative studies employing postcranial bones, the region is rarely utilised in qualitative systematic studies at less than the familiaral level and, where postcranial characters are used, they form only a limited proportion of the total character set: for example, in the review of the systematics of the Family Bovidae by Gentry (1992) only 35 of the 112 characters sampled were drawn from the postcranial skeleton; the remaining 78 characters were those of the skull, horns and dentition. There are very few studies that employ only postcranial bones; a notable exception is the work of Szalay on the morphology of the ankle joint in primates (1975) and marsupials (1982;1994) .Where postcranial characters are incorporated this is often a reflection of a frequent occurrence of postcranial material in the fossil record for the group concerned, as in the case of the bovids. Equally, the absence of postcranial characters from a study may reflect a paucity of this material in the museum collections used. This is a problem which afflicts even the largest of collections. For example, the Mammal Collections of the Natural History Museum, London, contain well in excess of 200 specimens of cuscus (genera *Ailurops, Strigocuscus and Phalanger*), yet in only four cases was the author able to find associated postcranial material. There is a good reason for this, namely that the conditions under which field collection of specimens is carried out may impede the collection of postcranial material. Given the fact that time and resources are often limited, complete dissection of the skeleton is often not feasible. A choice must then be made between preservation of the whole specimen in spirit (with the attendant problems of weight) or only part of the specimen, usually the skin (with tail and feet) and skull. In general, the latter option prevails, driven both by the necessity to reduce costs (in porterage and air freight) and the belief that postcranial material is of only limited use for systematic work. For this reason, postcranial material should be regarded as a rare and potentially valuable resource in museum collections.

The Skull. The skull is by far the most commonly used skeletal element in systematic studies of vertebrates. There are three main reasons for this. Firstly, the skull contains a large number of bones. Related to this is the fact the number of bones involved, and the complex patterns of development within the region, give rise to considerable scope for variability. However the third, and most likely reason is that the skull is the most commonly preserved bone complex in museum collections.

A wide range of potential character complexes exist within the skull. In mammals, these may be conveniently divided between three main regions.

1) The dentition. As Szalay (1994) points out, much of the mammalian fossil record is dental. This is particularly true for Mesozoic mammals and, since an understanding of the inter-relationships of these groups is vital for fixing the fundamental branching patterns of mammal phylogeny, it is perhaps unsuprising that judgements regarding taxonomic diversity and relationships are often based on dental characters. This is reflected in the complex nomenclature that has been developed to describe tooth morphology (figure 2). However, there is no a priori reason why dental characters should provide a better reflection of phylogenetic relationships than any other part of the animal's phenotype.

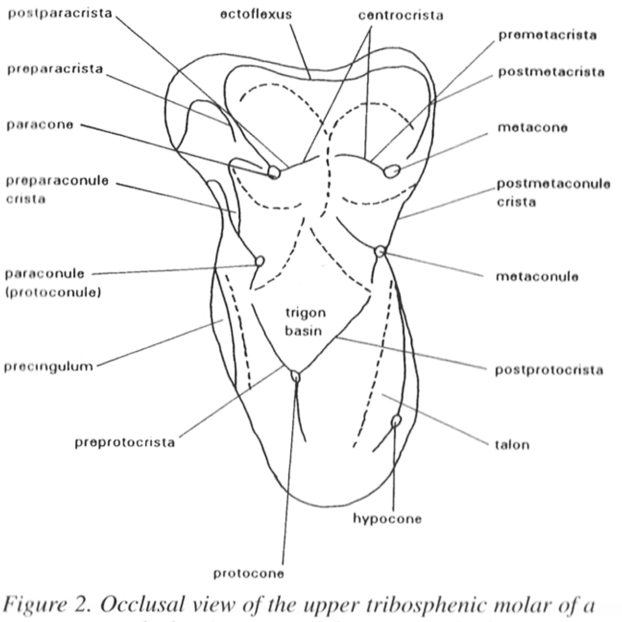

Figure 2. Occlusal view of the upper tribosphenic molar of a therian mammal, showing some of the cusps and other common features.

Teeth can be used in a number of ways for systematic studies. From a qualitative point of view, attention can be concentrated on the presence or absence of particular teeth, for example the premolars (always given that truly homologous teeth are being compared; Archer, 1975). Alternatively the morphology of the individual teeth can be studied, in terms of the presence or absence of particular cusps or ridges (cristae). Quantitative studies can look at the distances between teeth, the length of tooth rows, or the length of individual teeth. The latter is often difficult to measure directly, particularly in small mammals: a good way round this problem is, for example, to measure the length of the whole molar row (M1-4), then subtract the length of the row from the second to the last molar (M2-4) to give the length of M.

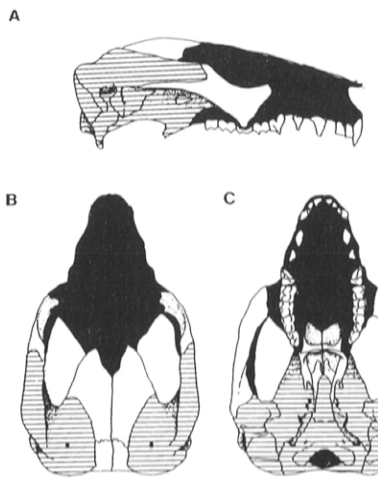

Figure 3. (A) lateral, (B) dorsal and (C) palatal views of the skull of a brushtailed possum (Trichosurus vulpecula). Dark shading = orbito-rostral region; horizontal shading = basicranial region.

2) The orbito-rostral complex. As the name suggests, this is basically the snout and the facial portion of the skull (figure 3), including the orbits. From the perspective of quantitative studies this is an extremely important area: the dimensions and proportions of the bones in this region are largely responsible for determining the shape of the skull. Qualitative studies in this area tend to concentrate on the relationships of the individual bones to each other, for example in the pattern of suture formation amongst the bones of the "orbital mosaic," which lies behind the eye (figure 4).

Figure 4. Orbital mosaics from (A) a marsupial (Didelphis), (B) an insectivore (Echinosorex) and (C) a primate (Lemur). L = lachrymal; F = frontal; A = alisphenoid; P = palatal. Shaded regions are cut surfaces of zygomatic arch (A, B & C) and postorbital bar (C only).

3) The basicranium. This is a region of great complexity, forming the underside of the braincase and including the jaw articulation and the bony structures of the middle and inner ear. Most of the major cranial nerves and blood vessels leave the skull through foramina in this region and the considerable variation in the branching patterns of these vessels leaves its mark in the variability in distribution of these foramina and their associated canals and sulci (grooves). Two of the most complex bony structures in the mammalian body, the periotic bone (which houses the cochlea and the semi-circular canals) and the auditory bulla (the bony floor of the tympanic cavity) are found in this region: both have been the subject of taxonomic studies (Novacek, 1977; MacPhee, 1981; Wible, 1991; Norris, 1993; 1994). Many of these structures are actually concealed within the skull and require dissection, although fragmentary specimens can prove useful in such cases.

Problems and Opportunities for Managers of Osteological Collections.

Given the revolution in the use of molecular techniques for systematic studies, it may seem surprising that there are still plentiful opportunities for the use of osteological characters in such studies. Ironically, however, the growing use of molecular techniques is likely to increase rather than reduce the demands made on museum collections. Phylogenies based on molecular data often conflict with established theories based on morphology, demanding re-examination of old morphological character states and the exploration of new character complexes (Novacek, 1992). It is in this climate of change, which Novacek optimistically describes as "a new renaissance" for morphological studies, that curators and collection managers will be faced with not only with great opportunities for the use of their collections, but also some important ethical dilemmas .

Destructive sampling. Extraction of DNA from bone for molecular studies requires the removal and destruction of part of the specimen. As was mentioned above, bone is not an ideal material for the extraction of DNA. For this reason, it is likely that such request is likely to be made in cases where osteological specimens are the only material available. Such cases could include specimens that are scarce in collections nationally, or where the species involved is either rare or extinct. Given that bone is a poor candidate as a source of DNA, it is probable that increasing the amount of the specimen removed will increase the chances of a successful extraction. Clearly, once a decision has been made to permit such destructive sampling, a successful outcome to the project is highly desirable if only because it makes it easier to justify the damage caused. For the person with responsibility for such material, a number of questions have to be asked. Is the project unique and important, or does it duplicate other studies? Are there alternative sources of material? If the decision is made to permit sampling, how much material should be removed? If the extraction is unsuccessful, should repeat sampling be permitted?

Dissection of specimens. Related to the issue of destructive sampling is the question of when to permit the dissection of osteological material. The constant drive for "new" character complexes for systematic studies means that the attention of taxonomists is increasingly being drawn to structures which are not visible externally. An example of such a character would be the periotic bone, which is one of the most substantial and complex bones in the mammalian skull and yet is barely visible externally in many groups of mammals. In such circumstances damaged specimens, in which the cranium has been smashed, can reveal many details of periotic structure. However, where such material is not available, partial dissection of the basicranial bones may be required.

Management practices. There is a growing realisation that analysis of character states in organisms involves not just an analysis of adult morphology, but also an understanding of the ontogeny of the character. In their early stages of development, skeletal characters are often membranous or incompletely ossified. Some characters may never fully ossify; for example the auditory bulla remains an entirely membranous structure in some mammalian taxa. Small bones may be suspended within such membranes, such as the taxonomically enigmatic class of bones known as entotympanics. Such features may be easily damaged, or even completely removed, by over enthusiastic cleaning of specimens. This is a factor that must be taken into account by collection managers, if they are not to dramatically reduce the utility of parts of their osteological collections.

Collecting and accessioning. Many of the requirements of the systematic researcher are addressed by a well thought out accessions policy. Clearly, it is vital that specimens come with good associated data, particularly where the collection may be used for quantitative studies (see above). Series of specimens from the same locality are also desirable, particularly where they build on existing strengths within the collection. There are other aspects which are perhaps less obvious at first sight. Even quite severely damaged specimens can prove useful for systematic studies where they reveal details of internal structures that would not otherwise be visible without dissection of the specimen. The collection and accessioning of postcranial material should also be considered as a priority: current practices may well be handicapping research workers, by leading to an overdependence on cranial characters.

Conclusions.

1) Museum osteological collections remain a valuable resource for systematic studies, whose usage is likely to increase with the increasing challenges presented by molecular studies. Bones are the only truly direct link between living species and the fossil record.

2) The usefulness of the collection is largely dictated by the research methodology to be employed. Quantitative studies are not easily undertaken in any but the largest of collections. Qualitative studies tend to require species coverage in breadth rather than depth. Molecular studies on osteological material should only be envisaged where bones are the only material available.

3) The majority of systematic work carried out on mammals concentrates on cranial characters. This is likely to be a reflection of the low numbers of postcranial material present in many museum collections.

4) The collections manager has an important role to play in increasing the utility of their collections for systematic studies, through the use of intelligent and proactive collection and accessioning policies and sensitive preparation, care and maintenance of specimens. In seeking to encourage the use of such collections for systematic studies, however, the collections manager will be required to address difficult ethical issues, particularly in relation to the dissection and destructive sampling of specimens.

References.

Archer, M. (1975). Abnormal dental development and its significance in dasyurids and other marsupials. *Memoirs of the Queensland Museum*, **17 (2)**: 251-265.

Diamond, J.M. (1990). Old dead rats are valuable. *Nature*, **347**, 334-335.

Golenberg, E.M., Giannasi, D.E., Clegg, M.T., Smiley, C.J., Durbin, M.,

Henderson, D. & Zurawski, G. (1990). Chloroplast DNA sequence from a Miocene *Magnolia* species. *Nature*, **344**, 656-658.

MacPhee, R.D.E. (1981). Auditory regions of primates and eutherian insectivores: morphology, ontogeny and character analysis. *Contributions to Primatology*, **18:** 1-282.

Menzies, J.I. & Pernetta, J.C. (1986). A taxonomic revision of cuscuses allied to Phalanger orientalis (Marsupialia, Phalangeridae). *Journal of Zoology, London*, **B1**, 551-618.

Norris, C.A. (1993). Changes in the composition of the auditory bulla in southern Solomon Islands populations of the grey cuscus *Phalanger orientalis breviceps* (Marsupialia, Phalangeridae). *Zoological Journal of the Linnean Society*, **107**: 93-106.

Norris C.A. (1994). The periotic bones of possums and cuscuses: cuscus polyphyly and the division of the marsupial family Phalangeridae. *Zoological Journal of the Linnean Society*, **111**: 73-98.

Novacek, M.J. (1977). Aspects of the problem of variation, origin and evolution of the eutherian auditory bulla. *Mammal Review*, **7**: 131-149.

Novacek, M.J. (1992). Mammalian phylogeny: shaking the tree. *Nature*, **356**, 121-125.

Paabo, S. (1989). Ancient DNA: extraction, characterization, molecular cloning and enzymatic amplification. *Proceedings of the National Academy of Sciences, USA,* **86**: 1939-1943.

Szalay, F.S. (1975). *Phylogeny, adaptation and dispersal of the tarsiiform primates.* In Luckett, W.P. & Szalay, F.S. (eds.), *Phylogeny of the Primates, a Multidisciplinary Approach.* New York: Plenum Press, 91-125.

Szalay, F.S. (1994). *Evolutionary History of the Marsupials and an Analysis of Osteological Characters.* Cambridge University Press.

Thomas, R.H., Schaffner, W., Wilson, A.C. & Paabo, S. (1989). DNA phylogeny of the extinct marsupial wolf. *Nature,* **340**, 465-467.

Thomas, W.K., Paabo, S., Villablanca, F.X. & Wilson, A.C. (1990). Spatial and temporal continuity of kangaroo rat populations shown be sequencing mitochondrial DNA from museum specimens. *Journal of Molecular Evolution,* **31**: 101 -112.

Wible, J . R. (1990) . Petrosals of late Cretaceous marsupials from North America and a cladistic analysis of the petrosal in therian mammals. *Journal of Vertebrate Paleontology,* **10**: 183-205.

THE OSTEOLOGICAL COLLECTIONS OF THE ZOOLOGY DEPARTMENT, LIVERPOOL MUSEUM.

Clemency Thorne Fisher, Curator of Birds & Mammals, Liverpool Museum, William Brown Street, Liverpool L3 8EN.

Introduction.

The osteological collections at Liverpool Museum amount to about 3,600 specimens, of which by far the greatest number are of mammals. Most of this material is housed in 36 wooden osteological cabinets, which were purpose built (many in-house) over several years (fig.1). They measure 220cm high, 93cm wide and 77cm deep and have a varying number of wooden drawers according to the height needed for the specimens stored. Eight of the cabinets are divided vertically down the centre so that they take half-width drawers (fig. 2); these were designed for the smaller specimens such as the birds and rodents.

Figure 1

There are also mammalian skulls kept with their associated skins in the study skin collection, which is now housed in metal cabinets. A few specimens of awkward size, such as a pair of champion African elephant tusks, are stored with the larger mounted mammals in a separate storeroom. All these cabinets and storage areas are on the Upper Horseshoe Gallery of the Liverpool Museum, but some osteological specimens are in use on the floor above by the Natural History Centre or are on display on the Natural History Gallery. One of our most famous specimens - the skeleton of Ambush II, the Prince of Wales' horse and the Grand National winner of 1900 - is on display in the Museum of Liverpool Life, next to the Maritime Museum on the waterfront. Ambush, who was genteelly flaking and who for some reason had had his real skull swapped with one of a zebra, was completely renovated for Liverpool Museum's Grand National Exhibition of 1989 and is now more suitably depicted with his original skull and in a galloping position (originally, he stood foursquare).

The osteology specimens can be summarized as consisting of one or more of the following sorts of material: antlers, horns, skeletons, skulls, loose mandibles, postcranial material without skulls, skulls with skins or mounts, teeth or tusks.

The small amount of human skeletal material that is held for comparative zoological reasons is stored in the same cabinet as other primates, but in separate clearly marked drawers. It is not used for general handling in places such as the Natural History Centre; replicas are used if required. These procedures are designed to satisfy the scientific and educational role of human material, whilst acknowledging the stated requirement of the Trustees of NMGM that we treat human remains with sensitivity.

Figure 2

Curation and Re-storage.

The curation and re-storage of the osteological collection has taken place over the last 20 years. In 1975 the collection was housed in a jumble of large cardboard boxes, in no particular sequence, on 'Dexion' racking covered with plastic sheeting making access impossible for either staff or visitors. The game heads (which were piled on the top rack) were the first to be removed, cleaned, mounted on plinths and then hung on racking; a position far less hard on their ears. They now hang in sequence, covered with a moveable canopy. They include mounted skulls and antlers as well as trophy skins.

As the new osteological cabinets became available, the specimens were removed from the large boxes bit by bit, each piece being cleaned, mended, identified and properly labelled (figs 3 and 4). Nearly all the specimens are now in