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# MUSEUM THERIOLOGICAL COLLECTIONS FOR THE STUDY OF GENETIC DIVERSITY

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ABSTRACT - Molecular methods to analyse DNA variability are opening new perspectives in the role played by museums in biodiversity research. DNA can be extracted from specific tissue collections, as well as from traditional voucher specimens. Ancient and museum DNA research produce valuable information for defining the phylogenetic positions of extinct *taxa*, the reconstruction of molecular and organismal evolution in extinct species, the characterization of extinct populations, including animal diets or microbial infections. Historical DNA samples are important sources of information also for conservation and evolutionary studies. In this paper, the methods used for ancient DNA analysis and the main results reported in published studies are reviewed.

*Key words*: Ancient DNA, DNA barcode, population genetics, phylogenetics, museum collections, genetic resources

RIASSUNTO - ***Le collezioni teriologiche museali e lo studio della variabilità genetica.*** I metodi di analisi del DNA aprono nuove prospettive per il ruolo dei musei nello studio della biodiversità. Il DNA può essere estratto da collezioni di tessuti, oppure dai tradizionali materiali museali. Le ricerche che utilizzano DNA antico e museale possono produrre informazioni utili per definire la posizione filogenetica di *taxa*, la ricostruzione dell'evoluzione molecolare e fenotipica di specie e la caratterizzazione di popolazioni estinte, incluse l'identificazione della dieta e la presenza di malattie infettive. L'analisi del DNA estratto da campioni storici può fornire informazioni importanti anche per ricerche di biologia della conservazione. Si analizzano i metodi utilizzati per l'analisi del DNA antico, corredati da un breve *excursus* dei risultati delle principali ricerche disponibili in letteratura.

*Parole chiav*e: DNA antico, DNA barcode, genetica di popolazione, filogenetica, collezioni museali, risorse genetiche

## INTRODUCTION

Green *et al*. (2006) suggested that Neanderthals and *sapiens* interbred in Europe before Neanderthals went extinct 30,000 years ago. In contrast, Wall and Kim (2007) analysed one million nucleotides of Neanderthal DNA showing that Neanderthals and *sapiens* did not interbreed, and that the results published in 2006 were based on samples contaminated with modern human DNA.

Wall and Kim (2007) reported that *c*. 80% of sequences used by Green et al. (2006) were from modern human DNA, not from Neanderthal. Thus, the controversy on the safe use of ancient

DNA (aDNA) is still continuing. The use of museum samples depend on the development of efficient methods for aDNA analysis. The first aDNA publications are just two decades old, but methods and standards of this new research field are evolving rapidly. As we have already seen, papers reporting erroneous results have fostered deeper investigations on DNA rates and patterns of degradation and long-term survival, and the development of methods for avoiding contamination and procedures for implementing authentication criteria of molecular data. The improving understanding of the effects of damages on aDNA templates is providing a robust framework for research. Two decades ago it was barely possible to genotype just very short fragments of mtDNA from relatively recent (late Holocene) specimens (Higuchi *et al*., 1984). Recently, entire mitochondrial genomes of extinct species, and even huge proportions of nuclear genomes have been sequenced through new research approaches named "metagenomics" (Poinar *et al*., 2006). Current research involving aDNA includes the definition of the phylogenetic positions of extinct *taxa* (Lister *et al*., 2005), the reconstruction of tempo and mode of molecular and organismal evolution in groups of extinct species (Baker *et al*., 2005), and the characterization of Pleistocene human and animal populations (Shapiro *et al*., 2004). The discovery of DNA preserved in ancient remains is providing information on a variety of issues, such as animal diets (Hofreiter *et al*., 2000), or microbial infections in extinct human populations (Zink *et al*., 2002). Ancient DNA is thus providing unique information to test a variety of assumptions in evolutionary and population genetics studies, and to reconstruct historical population and community dynamics. Historical DNA samples are important sources of information also for conservation and evolutionary studies. They allow us to reveal the evolutionary history of extinct species (Shapiro *et al*., 2002) and to assess loss of genetic variation in species with declining populations (Bouzat *et al*., 1998). Furthermore, samples collected over several generations from different populations might help to identify temporal dynamics of gene flow, genetic drift and selection (Nielsen *et al*., 1997). Initial results have revealed surprisingly complex population histories, and indicate that contemporary phylogeographic studies may give misleading impressions about the recent evolutionary past (Miller *et al*., 2006). Thus, museum collections of both traditional voucher specimens and genetic sources are entering in a new era.

## METHODS USED IN aDNA STUDIES

### 1. DNA degradation

Post-mortem DNA degradation is the main methodological problem in aDNA studies, because DNA degradation in unpreserved biological remains is unavoidable. Museums should implement efficient procedures to prepare and store safely contemporary biological samples for future DNA studies, aiming to minimize the processes of DNA degradation. However, aDNA recovered from ancient specimens preserved in museums or collected in nature, is always already damaged. It

would be interesting to fully understand the mechanisms of DNA damage, which could help to develop guidelines and methods to deal with damages. DNA damage in dead cells is generated by factors such as hydrolysis and oxidation. Consequently most specimens older than 1.000.000 year (but often older than just 100.000 years) do not contain any amplifiable endogenous DNA. Even for tissue samples less than 100.000 years old DNA is always degraded in fragments of *c*. 100–500 nucleotides (Paabo 1989; Handt *et al*., 1994; Hoss *et al*., 1996). Inter-strand cross-links in aDNA can prevent PCR amplification, or generate 'jumping PCR' artefacts (Geigl, 2002; Willerslev *et al*., 2004a). Post-mortem DNA degradation can generate additional and more subtle problems, such as strand breaks, baseless sites and miscoding lesions. These damages do not simply cause amplification failures, but might generate a variety of sequencing artefacts, and eventually lead to the preferential amplification of undamaged contaminant DNA (Paabo 1989; Lindahl 1993a; Hoss *et al*., 1996). The products of aDNA amplifications might cause sequencing artefacts, with a general bias toward CG|TA and AT|GC transitions due to a high rate of hydrolytic deamination of cytosine (or 5-methyl cytosine) to uracil and thymine (Gilbert *et al*., 2003a). Post-mortem DNA alterations may be so limited that the original mutation event can be identified and corrected (Gilbert *et al*., 2003b). In contrast, sometimes DNA lesions are very similar to those observed in regular evolutionary substitutions and cannot be identified. Although the dynamics of post-mortem damage is still poorly understood, several methods have been used to increase quantity and quality of DNA templates and sequence reliability. For instance, uracil-N-glycosylase (UNG) removes the deamination products of cytosine and is an important tool in testing the origin of sequence variation (Gilbert *et al*., 2003a). N-phenacylthiazolium bromide (PTB) appears to break intermolecular cross-links (Poinar *et al*., 1998). Similarly, high fidelity polymerase enzymes (Pfu and Taq HiFi) minimize sequence errors and increase amplification efficiency (Cooper and Poinar, 2001). Low temperature is highly beneficial to preserve the integrity of DNA molecules (Willerslev *et al*., 2004b). The oldest authenticated aDNA sequences are all from permafrosted tissues, including a more than 50.000 years old mammoth mtDNA (Hoss *et al*., 1994), a more than 65.000 years old bison mtDNA Shapiro *et al*., 2004) and Holocene and Pleistocene bones (Lambert *et al*., 2001). Other factors, such as rapid desiccation and high salt concentrations, may also help DNA survival (Lindahl, 1993b). However, small fragments of DNA (100–500 nucleotides) will survive for no more than 10.000 years in temperate regions and for a maximum of 100.000 years at colder latitudes (Poinar *et al*., 1996). Even under ideal conditions, amplifiable DNA cannot survive more than 1 million years. The oldest DNA sequences may exist in polar icecaps, with constant temperatures as low as –50°C.

## 2. The effects of ageing on DNA extracted from museum samples

Many studies have utilized dried museum specimens such as skins (Mundy *et al*., 1997), feathers (Taberlet and Bouvet, 1991) and bones (Wisely *et al*., 2004) of birds and mammals, and dried insects (Goldstein and DeSalle, 2003). Other groups, notably reptiles, amphibians, fish and aquatic invertebrates, have largely been preserved in alcohol and/or formalin, which cause much more difficult problems for DNA extraction (Schander and Halanych, 2003). However, independently of preservation methods, the quantity and quality of old DNA is strongly affected by ageing. Success rates of the polymerase

chain reactions (PCR), designed to selectively amplify the targeted DNA sequences, will always decline with the age of the DNA.

In population genetics studies, the amplification of microsatellite loci allows the use of these polymorphic markers to genotype historic samples and describe spatial and temporal changes in the genetic structure of historic and contemporary populations (Bouzat *et al*., 1998; Nielsen *et al*., 1997). However, scarce and degraded DNA may generate allelic dropout, that is, the stochastic amplification of only one of the two alleles at heterozygote loci, which results in erroneous genotypes. Wandeler *et al*. (2003) showed that the amount of DNA extracted from 279 red fox teeth samples collected between 1969 and 2000 was significantly affected by ageing. Although 73.8% of extracts contained sufficient DNA for reliable microsatellite genotyping, the quantity of DNA had decayed significantly over time in a non-linear pattern. The probability of a positive amplification for four examined microsatellites declined significantly with storage time ($P < 0.001$). Amplification performance depended on fragment-size, and there were significant interactions between time and size of the microsatellite alleles ($P < 0.001$), larger microsatellites having decayed more rapidly than shorter ones. As a consequence, the frequency of allelic dropout was higher for the older samples and for the larger alleles. These findings clearly indicate that, in museum DNA studies, PCRs should be designed to amplify fragments as shorter as possible, and always shorter than 100-200 nucleotides.

## 3. Long-term preservation of biological samples for DNA studies

Currently most tissue collections are frozen at *c*. - 15°C or - 20°C, or better deep frozen at - 80°C in electric freezers. Only deep freezing warrants long-term preservation of protein and DNA integrity. However frozen collections are exposed to the risks of electric power failures, and need an almost constant vigilance with alarm systems installed to avoid thawing and eventual loss of collections. A better, but more expensive system is to store tissues in cryogenic conditions, i.e. in liquid nitrogen (-120°C). The major advantage of liquid-nitrogen systems is that they increase the long-term stability of macromolecules, but samples are sometimes more difficult to access, which may be a problem for collections with very active loan and acquisition programs. Obviously, also liquid nitrogen levels must be regularly controlled and tanks need to be periodically refilled. Tissue culture methods have the advantage of providing an unlimited supply of genomic material but are labour-intensive to set up (see, for instance, the animal tissue collection maintained at the Center for Reproduction of Endangered Species, Zoological Society of San Diego).

Procedures for storage depend on the kind of macromolecules that should be preserved: RNA and enzyme activities are rapidly compromised, and tissue samples should be flash-frozen at – 80°C or, better, in liquid nitrogen. DNA in tissues and blood is more resistant, and samples could be stored safely for years also in normal electric freezers. In the field, flash-freezing of fresh tissues in liquid nitrogen, though logistically complicated, still represents the standard method for their preservation. High-molecular-weight DNA is very stable also at room temperature in ethanol or in a variety of lysis buffers (Seutin *et al.,* 1991), whilst specific protocols and storage buffers are needed to preserve RNA for PCR assays (Miller and Lambert, 2003).

Tissues and DNA samples are typically kept in test tubes or cryovials and organized in boxes and racks for easy retrieval. For sample tracking and mapping purposes, most large collections should complement traditional hand-written vial

labels with computer-generated labels or bar codes, which are permanent, easier to standardize, and less susceptible to degradation. Collections are usually organized taxonomically or numerically. A numerical organization scheme allows the rapid retrieval of tissues. Ideally, all or most of the samples should be vouchered with traditional specimens. However, also non-vouchered samples are valuable, if they have associated data. In some cases, such samples are associated with field voice recordings and/or photographs to increase their reliability. The term "e-voucher," coined by Monk and Baker (2001), applies to such documentation: "An e-voucher is a digital representation of a specimen…[it] may be ancillary to a classical voucher specimen or it may be the only representative of the specimen in the collection".

In conclusion, the method of preservation, both in the field and in the museum, should be selected such as to maximize the potential uses of the tissue, especially as specific techniques in genomics become more taxonomically widespread (Couzin 2002).

## 4. Procedures for aDNA extraction and amplification.

Standard procedures can be used to extract ancient and museum DNA, although laboratory organization should be anyway strictly monitored to avoid contamination and false positives (see below). However, aDNA extraction would certainly obtain better results if specific procedures were adopted such as *ad hoc* commercial extraction kits- e.g. the GeneClean for ancient DNA Kit (BIO 101, La Jolla, CA); QIAquick (Qiagen); DNeasy Tissue Kit (Qiagen). Most of these commercial kits are based on the silica method by Boom *et al*. (1990), which can be easily reproduced also using home-made solutions. The silica method is probably the most efficient and widely used method in aDNA research. Faster and cheaper methods, as the Chelex method (Walsh *et al*., 1991) are inefficient for aDNA extractions.

Standard PCR protocols can be easily improved for enhancing aDNA amplifications: it is imperative to use primers targeting short fragments (*c*. 100–200 nucleotides); the addition of anti-inhibitor molecules, like the bovine serum albumin (BSA), is strongly recommended; "hot start" PCR protocols can reduce the occurrence of non-specific annealing; standard number of PCR cycles (25-30) could be extended to *c*. 40-45 aiming to get more product; "nested PCR" (a primary PCR followed by a secondary one) can be carried out using the same or internal (nested) primers.

Duplicate extraction controls (no added tissue) as well as PCR negative controls (no template DNA) and positive controls (if possible) yielding PCR products of the correct size, should be routinely performed. However, the use of positive controls should be strictly controlled owing to the contamination risk that they pose.

Amplification products are assessed by electrophoresis on agarose gels. The independent replication of results by another laboratory is currently the strongest argument against laboratory-based contamination, because it is highly unlikely that the same erroneous sequence is independently obtained twice.

## 5. Biological samples used in ancient and museum DNA studies

Frequently aDNA is extracted from teeth and bone powder. Bones and skulls from vertebrate specimens in museums are often boiled within organic or inorganic solutions for preservation. The pulp of teeth is better protected from any external chemical action, often providing DNA of better quality. In juvenile samples, the larger amount of tissue within the pulp of growing teeth can improve the amount of

extracted DNA. There are two main extraction methods. A whole tooth is sealed in a zip-bag and frozen for 20 seconds in liquid nitrogen. After grinding the sample in a small steel mortar, the powder is transferred into a microcentrifuge tube and decalcified with EDTA buffer. The mixture is incubated under agitation at room temperature for 72 h. Then, samples are extracted. In a second procedure the tooth root is drilled, the pulp is collected into a test tube and directly extracted. Avian footpads are also extensively used, because they are likely to be less contaminated by the chemicals which are normally used to preserve skins and should produce better DNA. Small footpad fragments of *c*. 2-4 $mm^2$ are cut, their surface cleaned, and digested in prolonged lysis procedures (24-48 hours) using additional proteinase *K*. Hair roots and feather roots are standard samples for DNA extractions. They are usually extracted using silica-based procedures. Tissue fragments recovered from the inner side of the skin, feet, tail of skinned specimens can also be used, although the chemicals used for presservation could negatively affect the quality of DNAs. Small amounts of bone powder from sperm whale (*Physeter macrocephalus* ) teeth can be collected by drilling inside the root cavity, and yield sufficient DNA for sequencing without affecting the specimen's appearance.

## 6. The organization of the aDNA laboratory

Laboratory equipment and precautional procedures for safe aDNA manipulations must include: cleaning of surfaces with bleach; decontamination of chemicals and sample surface. All manipulations of specimens, extraction of DNA and PCR should be done in a laminar flow hood kept under short-wave UV irradiation when not in use, in a physically remote and dedicated laboratory. Furthermore, daily personnel movement should only proceed from ancient to modern laboratories. Additional precautions to eliminate contamination by contemporary DNA include regular decontamination of all surfaces and equipment (including pipettes) with 10% sodium hypochlorite solution; aliquoting and irradiation of test tubes with UV light; the use of specific protective clothing, equipment and reagents. Autoclaving does not prevent the amplification of short DNA fragments (up to 150 nucleotides), and often contaminates materials with bacterial DNA. Contamination is a main problem in laboratory procedures. Contamination can originate from a number of causes. Air movement created when opening PCR tubes or transferring liquids can create and disperse microscopic aerosol droplets, which can easily contain a million copies of the template. As a consequence, PCR products can quickly become widely distributed across laboratory surfaces, corridors and through entire buildings via personnel movement and air-handling systems. The most intractable problems occur when the sample itself has been contaminated prior to its analysis. This issue is of major significance with archaeological or museum materials, which have been handled by a variety of individuals who may have DNA markers close to or even identical to those of the specimen (Cooper 1997; Serre *et al.,* 2004). As many museum specimens have been handled for decades, contaminant molecules may also appear ancient and exhibit appropriate molecular behaviour, *i.e*. an inverse relationship between ampli-fication efficiency and fragment length (Handt *et al.,* 1994).

## 7. Markers used in aDNA studies

Markers used to genotype contemporary DNA samples can be used to type also historical samples. Mitochondrial DNA sequences are widely used to construct phylogenetic trees, identify species and, in

some cases, also the population of origin of the samples. Studies using aDNA utilize preferentially mitochondrial DNA markers, mainly because up to 1000 DNA copies per cell are available compared to single-copy nuclear DNA. Anyway, the use of sequences from nuclear genes is increasing. In particular, sex-linked sequences are used to identify paternal lineages in the populations. Microsatellite markers from aDNA samples are used to describe the extent of genetic diversity in past populations. Microsatellite data can be used to describe the genetic consequences of past bottlenecks, population expansions, the effects of drift in isolated populations and the rates of gene flow. However, due to the risk of errors in aDNA typing, criteria to authenticate any amplified DNA sequences should be applied, that is: negative controls, reproducibility and phylogenetic consistency (amplified sequences should show unambiguous affinities with other sequences in a phylogenetic analysis, and should be phylogenetically consistent with their supposed origins; Willerslev *et al*., 2004b).

## A REVIEW OF SOME PUBLISHED RESULTS

The first aDNA studies could obtain only very short sequences from museum skins of animals and humans (mummies, Higuchi *et al*., 1984; Pääbo 1985, 1989). These studies demonstrated that the genetic material surviving in ancient specimens was deeply contaminated by microbial or fungal DNA, and that endogenous DNA was generally limited to very low concentrations of short, damaged fragments of mtDNA. Advances in genetic analyses of aDNA were made possible by the invention of the polymerase chain reaction (PCR), which allows amplification and sequencing of even a few surviving endogenous molecules. However, the enormous amplifying power of PCR also created an increased sensitivity to contamination from modern DNA, and most of the first aDNA reports have been falsified. Some of these sequences originated from obvious human or microbial contamination, and it has not been possible to repeat independently the results. For example, aDNA has been reported from amber-preserved insects many millions of years old. Rigorous attempts to reproduce these DNA sequences have failed to detect any authentic ancient insect DNA. Lack of reproducibility suggests that DNA does not survive over millions of years even in amber, the most promising of fossil environments. Amber may not entirely protect DNA from decay as it is permeable to gases and some liquids and has prolonged contact with seawater during its formation (Poinar *et al*. 1996).

Studies which used more recent DNA from museum samples, however, got clearer and less controversial results. Losses of genetic diversity after recent population declines and extirpations have been well documented in several case-studies.

By the mid 20th century, the grey wolf (*Canis lupus*) was exterminated from most of the United States and Mexico. However, because wolves disperse over long distances, extant populations in Canada and Alaska might have retained a substantial proportion of the genetic diversity once found in the USA. Leonard *et al*. (2005) analysed mtDNA sequences of 34 pre-extermination wolves and found that they had more than twice the diversity of modern

wolves, implying a historic population size of several hundred thousand wolves in the western USA and Mexico. These results highlight the genetic consequences of population extinction within Ice Age refugia.

The arctic fox (*Alopex lagopus*) population in Scandinavia went through a severe demographic bottleneck in the early 20th century, and is today classified as critically endangered. Nystrom *et al*. (2006) used museum samples to investigate its pre-bottleneck genetic variation and compared it to modern samples from Scandinavia and North Russia. Variation in the mtDNA control region and five microsatellite loci was examined. Results showed that the arctic foxes in Scandinavia have lost approximately 25% of the microsatellite alleles and four out of seven mtDNA haplotypes. The results also suggest that the genetic differentiation between North Russian and Scandinavian foxes has doubled over the last 100 years.

The validity of subspecies designation has been assessed using museum samples from extinct populations. Miller *et al*. (2006) obtained mtDNA sequences from 108 brown bear (*Ursus arctos*) museum specimens. Phylogenetic analyses of historical and contemporary sequences were used to evaluate post-glacial colonization hypotheses, subspecific classifications, historical patterns and levels of genetic diversity in North America. In the past, the species occupied the western United States and northern Mexico but has been extirpated from over 99% of its range in the last two centuries. In mid-latitude North America a single moderately diverse clade is observed, represented by 23 historical haplotypes with up to 3.5% divergence. Only eight of 23 haplotypes (35%) are observed in the extensively sampled extant populations, suggesting a substantial loss of genetic variability. These data are consistent with recent genetic evidence that brown bears were south of the ice prior to the last glacial maximum. There is no support for previous subspecies designations.

Historical samples are well suited to document evolutionary responses to past climate changes. How species respond to an increased availability of habitat, for example at the end of the last glaciation, has been well established. In contrast, little is known about the opposite process, when the amount of habitat decreases. The habitat tracking hypothesis predicts that species should be able to track both increases and decreases in habitat availability (Dalen *et al*. 2007). The alternative hypothesis is that populations outside refugia become extinct during periods of unsuitable environmental conditions (Dalen *et al*. 2007). To test these hypotheses, Dalen *et al*. (2007) used aDNA techniques to examine genetic variation in the arctic fox (*Alopex lagopus*) through an expansion/contraction cycle. Their results show that the arctic fox in mid latitude Europe became extinct at the end of the Pleistocene, supporting the second hypothesis. Moreover, a high genetic similarity between the extant populations in Scandinavia and Siberia suggests an eastern origin for the Scandinavian population at the end of the last glaciation. These results imply that arctic species might be particularly

vulnerable to the increase of global temperature.

Molecular phylogenies reconstructed using historical samples are used to evaluate the rates of evolutionary changes. Prior to human settlement, 700 years ago, New Zealand had no terrestrial mammals (except for three species of bats), whilst approximately 250 avian species dominated the ecosystem. At the top of the food chain was the extinct Haast's eagle *Harpagornis moorei* (10–15 kg; 2–3 m wingspan), that was 30%–40% heavier than the largest extant eagle (the harpy eagle, *Harpia harpyja*), and hunted moa (*Dinornis maximus*) up to 15 times its weight. In a dramatic example of morphological plasticity and rapid size increase, Bounce *et al*. (2005) showed that *H. moorei* was very closely related to one of the world's smallest extant eagles, which is one-tenth its mass. This spectacular evolutionary change illustrates the potential speed of size alteration within lineages of vertebrates, especially in island ecosystems.

Phyogenetic analyses of aDNA from extinct species can help in resolving long-lasting controversies in systematics. The phylogenetic position of the giant deer, or 'Irish elk', showing huge antlers (the largest of any deer species, living or extinct), has remained an enigma. On the basis of its flattened antlers, the species was previously regarded as closely related to the living fallow deer (*Dama dama*). Recent morphological studies, however, have challenged that view and placed the giant deer closer to the living red deer or wapiti (*Cervus elaphus*). Lister *et al*. (2005) presented a new phylogenetic analysis, encompassing morphological and DNA sequence evidence, and found that both sets of data independently support a sister-group relationship between giant and fallow deer. These results include the successful extraction and sequen-cing of DNA from the extinct species, and highlight the value of a joint molecular and morphological approach.

Information from historical samples is valuable also in practical conservation biology. Bearded vulture (*Gypaetus barbatus*) populations in the Western Palearctic have experienced a severe decline during the last two centuries that has led to the near extinction of the species in Europe. Godoy *et al*. (2004) analysed the sequence variation at the mtDNA control region throughout the species range to infer its recent evolutionary history and to evaluate the current genetic status of the species. This study became possible through the extensive use of museum specimens to analyse populations now extinct. Phylogenetic analysis revealed the existence of two divergent mtDNA lineages, lineage A, occurring mainly in Western European populations, and lineage B in African, Eastern European and Central Asian populations. The relative frequencies of haplotypes belonging to each lineage in the different populations show a steep East–West clinal distribution with maximal mixture of the two lineages in the Alps and Greek populations. This phylogeographical pattern suggests allopatric differentiation of the two lineages in separate Mediterranean and African or Asian glacial refugia, followed by range expansion from the latter leading to two secondary contact suture zones in Central Europe and

North Africa. High levels of among-population differentiation were observed, although they were not correlated with geographical distance. Due to the marked genetic structure, extinction of Central European populations in the last century resulted in the loss of a major portion of the genetic diversity of the species. These results argue for the management of the species as a single population, given the apparent ecological exchangeability of extant stocks, and support the ongoing reintroduction of mixed ancestry birds in the Alps and the planned reintroductions in Southern Spain.

Past population dynamics have been documented using aDNA. The widespread extinctions of large mammals at the end of the Pleistocene have often been attributed to human persecution. Shapiro *et al*. (2004) used aDNA samples to reconstruct a detailed genetic history of the Beringian Steppe bison (*Bison priscus*) throughout the late Pleistocene and Holocene. Results depict a large diverse population living throughout Beringia until around 37.000 years ago, when the population's genetic diversity began to decline dramatically. The timing of this decline correlates with environmental changes associated with the onset of the last glacial cycle, whereas archaeological evidence does not support the presence of large populations of humans in Eastern Beringia until more than 15.000 years later.

Molecular procedures have been used by Hofreiter *et al*. (2000) to extract DNA from coprolites, excavated in Nevada and radiocarbon dated as 20.000 - 30.000 years old. Coprolites contained mtDNA sequences identical to that from a bone of the extinct Shasta ground sloth *Nothrotheriops shastensis*. Plant DNA sequences were also obtained from the coprolites and compared to homologous contemporary plant sequences. Thirteen families or orders of plants forming the diet of the Shasta ground sloth were identified, showing that it fed on trees as well on herbs and grasses.

## CONCLUSIONS

The development of improved techniques for the amplification and sequencing of ancient and museum DNA is leading museum collections to provide unique historical resources that can contribute significantly to novel population genetic, phylogenetic and systematic studies. Museum collections of inaccessible or extirpated populations of endangered or extinct *taxa* can represent an invaluable support also to conservation genetic studies and programmes. World-wide projects for typing DNA extracted from museum samples have been already launched. For instance, the Barcode of Life Data System (BOLD; http://www.barcodinglife.org/) is a web-based integrated bioinformatic platform, which supports specific research projects in the acquisition, storage, analysis and publication of DNA barcode records (Ratnasingham and Hebert, 2007). DNA barcoding consists in the sequencing of a standardized mitochondrial DNA (mtDNA) fragment that is used to drive species identification and new species discovery (Hebert *et al*., 2003; Savolainen *et al*., 2005). Studies on various groups of animals have shown that *c*. 700 nucleotides of the mtDNA *cytochrome*

*c oxidase* I gene (COI) is an efficient barcode sequence, which can lead to correct species identifications in more than 95% of the case-studies (Hebert *et al*., 2003, 2004a, 2004b). Among the many research projects launched within the BOLD, the DNA barcode data banks for all birds and fishes of the world are in progress. In the BOLD the DNA sequences are linked to voucher specimens, which can be physically located in museums, to other genetic data banks (*e.g*., the GenBank; http://www.ncbi.nlm.nih.gov/), and to distributional data (e.g., ORNIS; http://olla.berkeley.edu/ornisnet/; MANIS; http://manisnet.org/). BOLD aims to genetically characterize existing museum voucher specimens and help future field identifications and species discovery. Obviously, it is generally more difficult to generate barcode sequences from museum specimens that are more than a few decades old, since their DNA is degraded. However, genotyping museum specimens is useful, and in some cases, necessary. For instance, the validation of contemporary sequences collected from fresh specimens might need comparisons with the barcoding of their holotypes.

Sequencing historical samples might be necessary also for dissecting morphological cryptic species that are revealed only by DNA sequences (Janzen *et al*., 2005). Ancient DNA techniques can provide a means to determine which of the morphologically cryptic or homologous species correspond to the originally described type specimens (Austin *et al*., 2002), and to determine the phylogenetic positions of the holotypes.

Thus, the role of museums as repositories of specimens is developing for maintaining not only the morphological and sampling information, but also the integrity of biomolecules (proteins, DNA and RNA). Genetic sources collections, which typically consist of frozen tissues (heart, liver, muscle) and/or DNA collections, have now started to flank traditional voucher museum collections.

Tissue and DNA collections are opening new perspectives in the role played by museums in biodiversity research, but delineate also new responsibilities and issues. Sampling from genetic sources, being destructive and non renewable without further collecting, raises a number of issues regarding loan policies and reciprocation that are specific to these collections. Genetic source collections demand little space, but take substantial staff time to organize and are expensive to maintain. Loan activity can become a large investment for host institutions. Optimal use of biological samples will need efficient coordination of collection records (molecular biology-based data, digital images) via electronic and computer technology. Many more museum collections will be online in the future, and networking them could be facilitated by harmonizing procedures and developping collaborative standards. Coordination of existing collections and information will enhance the value and accessibility of collections and the awareness of the inventory of available tissues may stimulate the field collecting of missing specimens. Several preliminary efforts for a common

digital framework are in progress. Recent bioinformatics initiatives will ultimately link tissue-specimen collection records with bibliographic citations, competing taxonomic determinations, and geo-spatial referencing information with the aim of supporting genetic research.

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