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INVITED REVIEW

Ancient DNA applications for wildlife conservation

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Abstract

Ancient DNA analyses of historical, archaeological and paleontological remains can contribute important information for the conservation of populations and species that cannot be obtained any other way. In addition to ancient DNA analyses involving a single or few individuals, population level studies are now possible. Biases inherent in estimating population parameters and history from modern genetic diversity are exaggerated when populations are small or have been heavily impacted by recent events, as is common for many endangered species. Going directly back in time to study past populations removes many of the assumptions that undermine conclusions based only on recent populations. Accurate characterization of historic population size, levels of gene flow and relationships with other populations are fundamental to developing appropriate conservation and management plans. The incorporation of ancient DNA into conservation genetics holds a lot of potential, if it is employed responsibly.

Keywords: aDNA, climate change, conservation genetics, drift, endangered species, extinction, hybridization, wildlife management

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Introduction

The first report of the successful extraction and analysis of ancient DNA was from a single, century-old museum skin of the extinct quagga (Higuchi *et al*. 1984), and it triggered the application of ancient DNA techniques to a wide variety samples. Some spectacular claims of DNA from ancient plants, animals and bacteria up to hundreds of millions of years old followed. However, these very ancient materials were later shown to be incompatible with DNA preservation (reviewed in Cooper & Wayne 1998). Since these early papers were published, much more has been learned about DNA degradation, contamination and proper replication protocols, both theoretically and practically (Box 1, reviewed in Pääbo *et al*. 2004). Now, the number of studies fulfilling criteria for reliability is growing at an increasing rate each year. Ancient DNA studies in the past often involved one or few ancient samples that were compared to data from living animals to determine their relationships (i.e. Higuchi *et al*. 1984; Hänni *et al*. 1994; Krings *et al*. 1997; Cooper *et al*. 2001; Haddrath & Baker 2001; Orlando *et al*. 2003). However, as the practical issues have come under control, the application of ancient DNA techniques has expanded to include more diverse projects, including population level analyses (Thomas *et al*. 1990; Cooper *et al*. 1996; Hadly *et al*. 1998; Leonard *et al*. 2000, 2002, 2005a, b, 2007; Barnes *et al*. 2002; Hofreiter *et al*. 2002, 2004; Paxinos *et al*. 2002; Shapiro *et al*. 2004; Weinstock *et al*. 2005; Borge *et al*. 2007; Leonard & Wayne 2008).

The ability to genetically analyse past populations holds great promise for many lines of research, especially for conservation genetics. Conservation biologists' attention is often focused on threatened or endangered species. These often have reduced ranges and small population sizes that accelerate random genetic drift leading to rapid declines in genetic diversity and increases in population differentiation. These factors compound problems of determining how populations were in the past from the analysis of modern genetic diversity and patterns. The ability to directly measure the genetic diversity that was present in past populations enables the estimation of past population sizes, levels of gene flow, and relatedness between populations before the strong perturbations caused by humans in the last centuries or other discrete events (Ramakrishnan *et al*. 2005). These population parameters are critical to wildlife management and for setting conservation goals.

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Box 1 Ancient DNA: What? Where? When?

Ancient DNA is DNA extracted from old biological material, including (but not limited to) teeth, bones, museum skins, naturally mummified tissue and fish scales. These materials can originate from museum skins/skeletal material, archaeological material or paleontological remains and range in age from tens of years to about a hundred thousand years old (Lindahl 1993).

The DNA in all of these materials is degraded and is therefore more difficult to analyse than modern high quality DNA. DNA degradation leads to the three main problems: (i) reduced quantity of DNA which makes it particularly sensitive to contamination, (ii) broken DNA strands which reduce the length of fragments that can be amplified, and (iii) DNA damage which can lead to the identification of false mutations.

DNA damage and degradation is often more influenced by preservation conditions than by time (within approximately the last hundred thousand years). Some factors that favour DNA preservation include constant temperature, low temperature and dryness. A better understanding of these factors gives us more of an idea about the precautions necessary to obtain reliable ancient DNA results, although these factors are still not fully understood (reviewed in Pääbo *et al*. 2004). Systematic studies of the efficiency of particular extraction methods (Rohland & Hofreiter 2007) and polymerase enzymes (Stiller *et al*. 2006; Shapiro 2008) as applied to ancient DNA have revealed surprising differences between common methods, and more studies like these will benefit the field as a whole.

Isolation of ancient DNA extractions and PCR set-up from high quality DNA remains crucial. Proper negative controls in both extractions and PCRs, and appropriate replication of results are fundamental to any results based on old and/or degraded DNA. Many research groups are now overcoming these challenges and producing reliable, replicable data from ancient individuals and populations.

What to protect

Species are the unit of evolution and many conservation programmes revolve around them. Some very rare species from remote places are known only through a holotype. Validity of species based on a single specimen is generally called into question as they may represent an aberrant morphology of a known species, or a rare hybrid. Some examples are the long-billed reed warbler (*Acrocephalus orinus*) and Roosevelt's barking deer (*Muntiacus rooseveltorum*). A combination of ancient DNA analysis of the holotype and morphological or field data enabled the validity of both of the species to be confirmed (Amato *et al*. 1999; Bensch & Pearson 2002). Species diversity is an important component of the decision to create protected areas, so the existence of poorly known species such as these could be consequential.

Identification of distinct intraspecific units is generally more difficult, and more and more often relies on genetic data. Several efforts to define criteria for subspecific units have been made, such as evolutionarily significant units (ESU), management units (MU) (Moritz 1994; Crandall *et al*. 2000), and designatable units (DU) (Green 2005). They all include criteria involving genetic distinctness. However, modern populations may appear genetically distinct even though in the past they were connected through geneflow, due to the extinction of an intermediate population and/or recent drift.

Ancient DNA analyses of past populations are particularly important in cases where the same pattern in living populations could indicate recent drift or long-term differentiation. For example, Goldstein & DeSalle (2003) showed through ancient DNA analyses of historic northeast beach tiger beetles (*Cicindela dorsalis dorsalis*), a federally listed threatened subspecies, that the mitochondrial DNA haplotype identified as unique to Massachusetts was historically present across the northeast seaboard, although more frequent in the north end of the range. The 'north' haplotype was not lost in the southern end of the range or fixed in the northern end of the range until the very recent population crashes. This finding suggests gene flow across the region in the past, and thus supports re-introductions from adjacent areas. The strong impact of drift on genetic variation in very small populations suggests that this pattern may be common in many endangered taxa with currently isolated populations. For this reason, such studies are likely to be important in future management planning for a variety of taxa.

Ancient DNA analyses can also be used to confirm long-term differentiation. Some examples include the rare subspecies of the Sierra Nevada red fox (*Vulpes vulpes necator*) and the San Clemente Island loggerhead shrike (*Lanius ludovicianus mearnsi*) of California. DNA analysis of historic material showed that these subspecies were genetically differentiated from neighbouring subspecies and populations, even in the past (Mundy *et al*. 1997; Perrine *et al*. 2007). In these cases, ancient DNA was able to disprove the hypothesis that the differentiation seen in modern animals was only due to very recent drift in a small population. Since these subspecies belong to species that are numerous overall, demonstrating the long-term isolation is fundamental to their protection.

Specific conservation issues related to a particular population may also be informed by the genetic analysis of historic populations. For example, questions regarding the origin of the current Scandinavian wolf (*Canis lupus*) population raised by groups opposed to their existence suggested that the population was founded by illegally re-introduced zoo animals, and hence should not only not be protected, but should be eradicated. By combining ancient DNA analysis of historic specimens (Flagstad *et al*. 2003) with other data, Vilà *et al*. (2003) were able to confirm that the population did not derive from historic Scandinavian wolves. Instead, the authors demonstrated that the entire wild wolf population was founded by three individuals that colonized Sweden from Finland, and excluded the possibility that the founders originated from Scandinavian zoo stock. Hence, their legal protection was justified.

Wildlife management is subject to public opinion, policy and law as well as biological and other scientific observations and deductions. For this reason, innumerable individual and often local or culturally specific issues arise. Many of these issues, such as the example above, have to do with the origin of current populations. Ancient DNA analyses are particularly well suited to provide objective information in many of these cases, which is important in both courtrooms and the court of public opinion.

Captive breeding, re-introductions and augmentations

Captive breeding programmes can be an important component of the management of some endangered species or subspecies. Determining which individuals should be included in captive breeding programmes while avoiding both inbreeding and outbreeding depression is a major challenge (Edmands 2007). There are some examples of species that have passed through severe bottlenecks in which ancient DNA analyses demonstrated that most of their genetic variation was lost, but were still able to increase in population size once direct persecution ended, such as the Northern elephant seal (*Mirounga angustirostris*, Weber *et al*. 2000; Hoelzel *et al*. 2002). Although the population size has increased greatly, morphological evidence of inbreeding depression (increased bilateral asymmetry) was identified in postbottleneck/genetically depauperate individuals (Hoelzel *et al*. 2002). Another example of a population with a small number of founders, which increased in size rapidly despite very low levels of genetic variation is the Scandinavian wolf population which has increased to over a hundred individuals from just three immigrants well differentiated from the historic population (Vilà *et al*. 2003). Despite the demographic expansion of this population, Liberg *et al*. (2005) have shown inbreeding depression leading to lower survival of pups, and a morphological analysis has revealed an increase in the number of skeletal abnormalities compared to the source population (Räikkönen *et al*. 2006). Populations such as this continue to depend on genetic rescue by immigrants that would reduce the impact of inbreeding.

The problems associated with a reduced number of founders are commonly faced by captive breeding programmes as well as by wild populations. A further complication for captive breeding programmes for endangered species is that founding individuals may already be depleted of genetic variation. One example where ancient DNA from historic specimens proved valuable to a captive breeding programme is that of the European common hamster (*Cricetus cricetus*). Populations of Western European common hamsters crashed in association with changes in agricultural practices, and are now highly endangered in several Western European countries. Through genetic analyses of recent hamsters from Eastern and Western Europe and historic animals from Western Europe, Smulders *et al*. (2003) showed that the extremely reduced amount of genetic variation in living Western European hamsters is a result of the bottleneck in the last few decades. Their analyses of historic specimens further enabled them to determine that Eastern and Western European populations were connected through high levels of gene flow in the recent past. From this they determined that it would be appropriate to augment the genetic variation present in the captive stock with animals from Eastern Europe, where they remain numerous and genetically diverse. Most captive breeding programmes are very sensitive to the possibility of inbreeding and take action to minimize this acknowledged danger. Employing ancient DNA analyses to identify all possible sources of founders could potentially make this job somewhat more tractable.

Another issue that can threaten a captive breeding programme is verity of the founders. If the founders do not represent the taxa that managers are trying to conserve through captive breeding, the entire effort may be for naught. Wayne & Jenks (1991) showed that it is possible that the red wolf (*Canis rufus*) had a hybrid origin, and these data have been used to argue against its status as 'Endangered'. However, through the ancient DNA analysis of more historic specimens, Roy *et al*. (1996) were able to show that the founders of the captive breeding programme did represent the historic population of wild red wolves, and thus supported their continued protection. If any questions regarding the identity of the founders exists when a captive breeding programme is set up, or later in response to controversy, ancient DNA analysis may be the only way to ascertain the genetic purity of an individual or population. In some cases, the inclusion of nonpure animals may be necessary or determined to be most beneficial in the long term, but at least that decision will be an informed one.

Re-introductions of animals to areas where they were historically extirpated do not always involve captive-bred animals. Translocations of wild animals from other areas may also be used. Genetic appropriateness of re-introduced or translocated animals to their new home is always of critical importance (e.g. as shown for the ibex, *Capra ibex*, Greig 1979). Ancient DNA analyses of past populations may assist in the identification of appropriate animals by measuring the overall similarity of the past population to those individuals available to potentially participate in the re-introduction. For example, the ostrich went extinct in Saudi Arabia during historic times. As re-introductions were being considered, Robinson & Matthee (1999) analysed the mitochondrial DNA sequences from two historic Saudi Arabian ostriches, and compared them to mitochondrial DNA sequences from all extant subspecies of ostrich in order to determine which extant populations were most closely related to the extinct population. Their DNA sequences showed that the population of ostriches selected for re-introduction based on ecology was also the most closely related to the extinct population, and thus supported the plan. Although genetic relatedness is not generally sufficient by itself to determine appropriateness of a source population, it may be an important contributing factor.

The range of some species just tens to hundreds of years ago may have been very different than they are today. For some endangered species, uncovering the past range may be important for recovery plans because it may expose the existence of more suitable habitat available for re-introductions. For example, ancient DNA analyses of morphologically unidentifiable osteological remains from the main Hawaiian islands revealed the presence of Laysan ducks (*Anas laysanensis*) on islands other than Laysan, laying the foundation for their widespread introduction across the Hawaiian islands (Cooper *et al*. 1996). The establishment of multiple populations is important to the long-term survival of this and other species that exist as a single population, because this is the main way to reduce their probability of extinction due to stochastic environmental factors.

Local extirpations may lead to the erosion of genetic diversity of a species (Leonard *et al*. 2005a; Barnett *et al*. 2006). Sometimes, however, animals collected from these regions or their descendents remain in living collections (i.e. zoological parks). Accurate identification of these individuals could lead to re-introductions and recovery of genetic variation important for long-term survival of the species in the wild. Genetic characterization of the past population is a prerequisite for properly identifying individuals in living collections appropriate for re-introduction. Such projects have been undertaken for Galápagos land iguanas (Hofkin *et al*. 2003) and North African Barbary lions (Barnett *et al*. 2006). Re-introduction of genetic material lost in wild populations but surviving in living collections may be an important safety net for genetically depauperate populations. Ancient DNA analyses of past populations in combination with genetic analyses of postaugmentation populations can then used to determine if the re-introduced individuals or genetic material were integrated in the population (Jacobsen *et al*. 2008).

Biological invasions, introductions, and hybridization

Recent habitat changes and human-mediated introductions sometimes put in contact closely related but naturally allopatric species that have not evolved either intrinsic or behavioural barriers to reproduction. Hybridization between native and introduced taxa can have significant impact on the native species, including extinction (Perry *et al*. 2002). Once hybridization starts, it can be very difficult to determine how severe an impact it has had, especially if the taxa are closely related. This can threaten species across many taxonomic groups, including invertebrates (Perry *et al*. 2002), fish (Perry *et al*. 2002), birds (Mank *et al*. 2004; Muñoz-Fuentes *et al*. 2007), mammals (Roy *et al*. 1996; Leonard & Wayne 2008) and plants (Saltonstall 2002). Ancient DNA evaluation of historical specimens is therefore fundamental to unravelling the history of the population and to quantifying the amount of introgression.

Through a combination of ancient and modern DNA analyses, Mank *et al*. (2004) showed a breakdown of species integrity between black and mallard ducks (*Anas rubripes, A. platyrhynchos*) in North America over the course of just 60 years. A similar situation has been revealed for the Great Lakes area wolves (Leonard & Wayne 2008). Genetic characterization of the species before hybridization and introgression may pave the way for identifying individuals with different levels of genetic introgression (pure, F_1 hybrid, backcross, etc.). This data can be used to emphasize the breeding of pure individuals (i.e. red wolves, Adams *et al*. 2003) or to cull hybrids (i.e. white-headed duck, *Oxyura leucocephala*, Muñoz-Fuentes *et al*. 2007).

Evaluation of human impact

The management strategies of culling and stocking are employed specifically to reduce or increase the numbers of a population. The intention of most control and stocking programmes is not to eradicate or replace wild populations, but to hold them at a particular different size, often for recreational hunting and fishing. The impact these strategies have on populations can vary, even within the same species, and may be heavily dependent on the initial size and genetic diversity of the populations. In a study of the impact of stocking on the brown trout (*Salmo trutta*) populations in two different rivers, Hansen (2002) used ancient DNA to compare prestocking populations with the population used for stocking and the current populations. Hansen identified strong selection against the stocked trout. However, in the case where the breeding population was very small at the time of stocking, introgression was much more extensive than in the larger population. Although data from only recent animals had indicated some introgression, the ancient DNA analyses of prestocking populations uniquely allowed

Hansen to identify pure native fish to use in the supportive breeding programme. Ancient DNA analyses can thus be used to evaluate the impact of management strategies. Further studies along these lines should help determine what the consequences of different management actions are, and thus lead to improved management.

Hunting and fishing (both recreational and commercial) may impact the genetic structure of populations in unexpected ways. Studies designed to determine the impact of fishing on genetic population structure across space have employed ancient DNA analyses of historic specimens in combination with recent animals in a variety of heavily fished stocks and have reached apparently different, sometimes even opposite, conclusions. Most of the fish stocks surveyed maintained stable population structure through time (Atlantic salmon, *Salmo salar*, Nielsen *et al*. 1999; Tessier & Bernatchez 1999; Newfoundland cod, *Gadus morhua*, Ruzzante *et al*. 2001), but some change in population structure through time was identified in northern British Columbian steelhead trout (steelhead trout, *Oncorhynchus mykiss*, Heath *et al*. 2002). A stable geographical population structure may indicate that the species is highly phylopatric, in which case if one population is lost it may be very difficult for it to be naturally re-established. It may also indicate local adaptation that could be undermined if the population structure was disturbed. However, populations may be simultaneously impacted by multiple important factors, such as hunting/fishing and climate change. Further studies, preferably incorporating multiple factors and populations, will be needed to determine what causes shifts in populations through time, if it is detrimental, and if so, how to mitigate the causal factors.

The results from ancient DNA studies regarding the impact of fishing on genetic diversity are clearer. For example, Hauser *et al*. (2002) were able to genetically analyse scales from two populations of New Zealand snapper (*Pagrus auratus*) across the entire time of exploitation for one of them. Despite vary large population sizes in the tens of millions, Hauser *et al*. found that genetic diversity eroded through the 50 years of exploitation. Since loss of genetic diversity could lead to reduced adaptability, population persistence and productivity, it is important to adjust fishing strategies to minimize genetic as well as demographic impact. Many studies have shown a reduction in genetic variation through time in wild populations (see Table 1), although endangered populations are the ones that have attracted the most interest from researchers. More studies on the changes of genetic diversity through time in exploited and not endangered populations could be particularly informative.

Escapees from managed populations (i.e. fur and fish farms, wildlife exhibits and pets) can sometimes survive in the wild and interbreed with local related species and in doing so threaten their persistence in a variety of ways, including hybridization (see above) and disease transmission. Coughlan *et al*. (2006) used ancient DNA to survey both neutral microsatellite loci and one locus tightly linked to the immune response gene Satr-UBA (MHC I), in a wild sea trout (*S. salar*) population before and during aquaculture activities. They found a dramatic decrease in the variability in the immunologically important locus without impact to the neutral loci. This suggests that diseases carried by the farmed animals impacted the wild populations. This observation should inform decisions about when, where and how aquaculture should be practiced. It also suggests that much more ancient DNA work on functional nuclear loci could be practical and informative, as illustrated by some recent studies (Jaenicke-Després *et al*. 2003; Newcomb *et al*. 2005; Römpler *et al*. 2006; Svensson *et al*. 2007).

Connectivity management

Many previously widespread species have had their range reduced historically due to anthropogenic impact including direct persecution and changes in land use. This has left small, geographically isolated remnant populations. These remnant populations may appear very genetically differentiated from conspecific populations due to the strong effect of genetic drift (Vilà *et al*. 2003; Muñoz-Fuentes *et al*. 2005; Martínez-Cruz *et al*. 2007). Small populations and populations with very low genetic diversity are also at risk for inbreeding depression (Edmands 2007). One extreme example of the impact of inbreeding is the Florida cougar. They suffered greatly reduced fitness due to deformed sperm, abnormalities in their hearts, and other morphological and immunological problems (Roelke *et al*. 1993). A combined analysis of ancient and recent animals determined that the historic North American population was genetically homogeneous (Culver *et al*. 2000). This ancient DNA analysis of historic material provided further support for translocation of cougars into Florida from adjacent, but currently isolated, populations of cougars for the purpose of introducing genetic variation.

Even if no inbreeding depression is observed, a lack of genetic diversity may reduce the evolutionary potential of a population to adapt to the changing environment (Booy *et al*. 2000; Pujol & Pannell 2008). So, if an isolated population was previously connected to other populations, it may be important to re-establish gene flow to maintain the long-term viability of the population (Miller & Waits 2003). However, genetic differentiation between populations may indicate that the population has been isolated for a long time, and may have evolved important local adaptations. In this case, establishing gene flow could lead to outbreeding depression, and the breakdown of locally adaptive suites of traits/alleles that could further endanger the population (Edmands 2007).

Ancient DNA analysis of historic populations, both from the localities now isolated and from intermediate areas where the species has been extirpated, can help disentangle these alternatives. For example, the white-headed duck was

Table 1 Historic declines in genetic diversity of wild populations identified through ancient DNA analysis

Species	Marker	Reference(s)
Birds		
Bearded vulture *Gypaetus barbatus*	mt	Godoy *et al*. (2004)
Greater prairie chicken *Tympanuchus cupido*	ms	Bouzat *et al*. (1998)
Mauritius kestrel *Falco punctatus*	mt	Groombridge *et al*. (2000)
Nene *Branta sandvicensis*	mt	Paxinos *et al*. (2002)
Spanish imperial eagle *Aquila adalberti*	mt, ms	Martínez-Cruz *et al*. (2007)
White-headed duck *Oxyura leucocephala*	mt	Muñoz-Fuentes *et al*. (2005)
Whooping crane *Grus americana*	mt	Glenn *et al*. (1999)
Fish		
New Zealand snapper *Pagrus auratus*	ms	Hauser *et al*. (2002)
Sea trout *Salmo trutta*	MHC	Coughlan *et al*. (2006)
Mammals		
Arctic fox *Alopex lagopus*	mt, ms	Nyström *et al*. (2006)
Black-footed ferret *Mustela nigripes*	ms	Wisely *et al*. (2002)
Brown bear *Ursus arctos*	ms, mt	Miller & Waits (2003), Miller *et al*. (2006)
Common hampster *Cricetus cricetus*	mt, MHC	Smulders *et al*. (2003)
Gray wolf *Canis lupus*	ms, mt	Flagstad *et al*. (2003), Leonard *et al*. (2005a)
Hairy-nosed wombat *Lasiorhinus krefftii*	ms	Taylor *et al*. (1994)
Hector’s dolphin *Cephalorhynchus hectori*	mt	Pichler & Baker (2000)
Northern elephant seal *Mirounga angustirostris*	mt	Weber *et al*. (2000), Hoelzel *et al*. (2002)
Sea otter *Enhydra lutris*	mt, ms	Pertoldi *et al*. (2001), Larson *et al*. (2002)

mt, mitochondrial DNA sequences; ms, nuclear microsatellites; MHC, immunohistocompatibility locus, a nuclear locus important for immune response.

historically widely distributed from Spain to western China. Populations around the Mediterranean have been decimated in the last century, and there are now few, isolated populations left in Spain, Greece and two in the east. One of the larger populations is in Spain, where they have rebounded from about 22 individuals in the 1970s to a few thousand today, although genetic diversity is extremely low (Muñoz-Fuentes *et al*. 2007). Muñoz-Fuentes *et al*. (2005) studied the historic and modern genetic diversity of the white-headed duck from across its range and showed that the severe historic bottleneck reduced the level of genetic diversity in the Spanish population. Perhaps more important for long-term conservation of the species is their finding that before the recent bottlenecks and local extirpations, there was no genetic structure across the range of the species. This opens the possibility to reinforce genetically depauperate populations, such as Spain, with ducks from other populations, as well as to use the numerically more substantial Spanish population as a source to reintroduce the species in areas around the Mediterranean where it went extinct. Ancient DNA data have also been used to support the active management of gene flow in Yellowstone brown bears (Miller & Waits 2003) and bearded vultures (Godoy *et al*. 2004). Understanding past levels of gene flow and considering active management in order to re-establish that gene flow across potentially large areas of unsuitable habitat is important for many species. This is perhaps especially important for top predators, which have low population densities, and thus it will be difficult to maintain sufficient numbers of individuals for a healthy population in many parks and wilderness areas.

Population sizes

Historic effective population size may be estimated from genetic diversity in populations and be used as a scientific basis for conservation targets, as has been carried out in several species of whales (Roman & Palumbi 2003). However, the extremely reduced genetic diversity often observed in endangered species undermines such analyses (Table 1). Genetic analysis of ancient material may provide a better estimate of the past effective population size than estimates based on recent data alone. For example, the ancient DNA analysis of gray wolves from North America where they have recently been extirpated identified more genetic diversity than had been identified in living North American animals (Leonard *et al*. 2005a). This is likely due to the eradication campaigns disproportionately affecting glacial refugial populations. Taking into account the genetic diversity of historic gray wolves, the estimated historic population size of gray wolves in western North America was several times greater than the extant population. These data can help put conservation goals into perspective by allowing them to be compared to the historic state. Such estimates based on scientific data are important because in the absence of scientific evidence to support a particular population size goal, economics and politics may dominate in species recovery plans (Povilitis *et al*. 2006).

Climate change

Current rapid climate change has already begun to impact the biology of many species (Bradshaw & Holzapfel 2006). Some understanding of how species are reacting and will react to environmental change is fundamental to long-term conservation planning. Determining how past populations dealt with environmental changes may help managers predict how species and populations today will be impacted by ongoing environmental changes, and therefore plan for their future needs, as well as their present ones. A few ancient DNA studies have directly addressed how single populations have evolved to adapt to rapid climate change over thousands of generations (Hadly *et al*. 1998; Hofreiter *et al*. 2004; Dalén *et al*. 2007; Leonard *et al*. 2007).

A combined analysis of ancient DNA and morphology showed that as climate changed through the Holocene, a gopher population in Yellowstone did not track its preferred habitat, but stayed put and rapidly changed in morphology (size) to adjust to changing conditions (Hadly *et al*. 1998). Ancient DNA studies of Pleistocene mammal populations in Europe also showed that populations of a variety of mammal species did not track suitable habitat as the climate changed (Hofreiter *et al*. 2004; Dalén *et al*. 2007; Valdiosera *et al*. 2007). When conditions become too far outside their niche in the late Pleistocene, those populations went extinct. These studies suggest that the rapid behavioural, developmental and morphological adaptations currently being observed in response to the ongoing global warming (Bradshaw & Holzapfel 2006) may hit a point at which adaptation is no longer possible, and a number of extinctions may occur.

A better understanding of risk of extinction for populations will hopefully help us preserve those being impacted by current climate changes. An extensive study involving morphology and isotope analyses as well as ancient DNA analyses on gray wolves in eastern Beringia (Alaska) identified a specialized ecomorph that hunted Pleistocene megafauna, many of which went extinct at the end of the Pleistocene (Leonard *et al*. 2007). This Pleistocene wolf itself then went extinct at the end of the Pleistocene and consequently is not ancestral to the living population in Alaska. This study highlights the increased risk of extinction associated with specialization, not only at the species level, but also at the population level. These data also illustrate how important multiple populations are to species survival. More studies such as these and others on brown bears (Leonard *et al*. 2000; Barnes *et al*. 2002) that track populations through the end-Pleistocene and Holocene climate changes may be able to more precisely determine which factors trigger adaptation, and which cause populations to go extinct.

Forensics

Laws made by individual countries, such as the Endangered Species Act (ESA) in the USA, and international treaties, such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the International Convention for the Regulation of Whaling (ICRW), are important tools for conservation. However, enforcing these regulations can be very difficult because once animal products have been processed it may be very difficult to tell apart products derived from legally vs. illegally obtained species or individuals. The same processing which may alter the morphology to the point that it is unidentifiable may also damage the DNA so that it is very difficult to analyse. The application of ancient DNA techniques to these materials, even though their age is not great, is required to obtain reliable, replicable genetic data. Despite the difficulties, genetic analysis of wildlife products has been successful in many cases (Baker & Palumbi 1994; Malik *et al*. 1997; Baker *et al*. 2000; Shivji *et al*. 2005). Such analyses enable the rigorous enforcement of conservation laws necessary for them to be effective.

The future of ancient DNA in conservation genetics

The potential for the application of ancient DNA techniques to conservation and management issues is huge and is just beginning to be exploited. The number of research groups employing ancient DNA techniques is growing rapidly, as techniques for retrieval of DNA from historic specimens have been refined. A better understanding of where ancient DNA can be found, basic precautions against contamination, and the optimization of several good DNA extraction methods for ancient DNA (i.e. Rohland & Hofreiter 2007), improvements in technology such as modified *Taq* enzymes (i.e. Ampli*Taq* Gold by PerkinElmer and HotStar by QIAGEN) and potentially specially evolved *Taq* (Shapiro 2008) and the ability to sequence very short polymerase chain reaction (PCR) products (i.e. pyrosequencing by Biotage) have all helped make ancient DNA more accessible.

Genomic approaches are also supporting this surge in interest. The availability of huge quantities of genetic data from a variety of species enables the construction of conserved primers that may be usable in nonmodel organisms. Depending entirely on markers developed in recent samples can introduce ascertainment bias. Variation present in living population(s) may be identified or not in past population(s), but variation that was present in the past but is no longer extant will be missed. For this reason, applying genomic screening directly to ancient populations or individuals of interest is also important. The creative application of genomics technology, such as 454 DNA sequencing (Roche Applied Sciences), Solexa (Illumina) and SOLiD (Applied Biosystems) or other large-scale sequencing technologies on the verge of being available, are enabling ancient DNA to be directly analysed. For example, Meyer *et al*. (2008) have devised an alternative protocol for one of the vital preparation steps for high-throughput sequencing, library quantification. In doing so they have reduced the amount of starting DNA necessary for a successful 454 sequencing run several orders of magnitude to something reasonable with ancient samples, and have reduced the cost of such analyses.

High throughput sequencing yields a large number of short random sequences, but the high cost of each run is prohibitive for many projects. Further, if a 1/16 run in a 454 sequencing instrument (approximately 12 000 reads) was carried out on each of several individuals, the amount of alignable sequence between them may be very disappointing. In order to increase the amount of comparable data obtained from each run, pre-amplifications with short random primers (such as amplified fragment length polymorphism primers) can be made. The range of appropriate length fragments can then be excised from a gel and used as the basis for the high throughput sequencing run. Such a strategy in combination with nucleotide barcodes (Parameswaran *et al*. 2007) can further increase the yield of informative data by negating the need for physically partitioning the sequencing plate, which reduces the number of reads (i.e. 16 runs of 1/16 plate yields approximately 192 000 reads, and a whole plate yields approximately 440 000 reads on a 454 sequencing instrument). Multiplexing with nucleotide barcodes can also be employed to use high throughput sequencing technology to sequence a large number of targeted PCR-amplified regions (Binladen *et al*. 2007).

The era of genomics should also enable ancient DNA practitioners to move beyond neutral molecular markers and actually track functional genes through time. It is the change in the functional genetic diversity that we need to understand because that, after all, is the fodder of evolution so important to the survival of species. Many functional genes are being identified, and using ancient DNA it is possible to track how these potentially important genes have evolved through time (i.e. Jaenicke-Després *et al*. 2003; Newcomb *et al*. 2005; Svensson *et al*. 2007). Tracking functional variation, including protein coding genes and noncoding regulatory regions, through time in wild populations could complement and extend the application of ancient DNA to conservation in almost all of the topics covered in this review. It could be especially important for more accurately estimating the impact of drift. A better characterization of how drift is impacting the genome could better enable both inbreeding and outbreeding to be avoided in captive and wild managed populations.

More and more individual researchers see how DNA analyses of historic and prehistoric populations can complement their ongoing lines of research. The large-scale incorporation of these techniques into a wide variety of projects should reveal interesting new aspects of the biology and history of many species and populations, and contribute to their conservation. These data not only contribute to conservation by furthering basic knowledge of the biology and ecology of species, but should yield scientifically supported concrete conservation and management recommendations. By the time some populations or species are of conservation interest, there may be so few individuals left in them that there are few chances to understand their evolutionary history and the factors that dictated their fate. The creative employment of ancient DNA techniques enables hypothesis testing by opening the past to DNA analyses (i.e. Paxinos *et al*. 2002). These can have concrete results such as recommending sites to establish new populations (i.e. Cooper *et al*. 1996), recommending that populations be conserved separately (i.e. Perrine *et al*. 2007) or that active management to create gene flow is appropriate (i.e. Miller & Waits 2003). These are examples of species-specific recommendations, but ancient DNA can also be employed to determine how specific management actions impact populations (i.e. Hauser *et al*. 2002). Conservation and management recommendations based on rigorous science will yield the best results, and will also stand up both in courts of law and courts of public opinion, both crucial to the success of most conservation efforts.

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Jennifer Leonard combines genetic and other data to determine how and why populations are structured through time and space, and which factors drive some populations to extinction or expansion.