Looking backwards to look forwards: conservation genetics in a changing world

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Introduction

The current consensus is that the world is rapidly warming, and that the anthropogenic production of greenhouse gases is a major contributing factor. There are, of course, other ways in which environments change, sometimes anthropogenic (especially habitat loss and fragmentation), and sometimes not, but climate change in particular has our attention at the moment. In the context of climate change, mobile species that are not prevented from moving by physical barriers may simply move to where the climate is more suitable (e.g. Root et al. 2003; Gregory et al. 2009). There are a number of studies that model the projected pattern of re-distribution, and a recent paper describes the implications for designing and maintaining wildlife reserves (Hole et al. 2009). In general, a strategy for reserve design would need to emphasize the importance of corridors and stepping stone habitat to provide the capacity for redistribution in future as climate changes. Of course there will be practical and logistical problems associated with this. For example, since multiple species will be affected, likely including those that are interdependent (e.g. predator and prey), the outcome will be hard to predict. It's also not trivial to predict where populations may move, which is typically based on current species distributions and projected changes in 'climate envelope' (Pearson and Dawson 2003).

Another possible response to changing environments is local adaptation to the new conditions. There are many examples, perhaps the most famous of which is the rapid evolution of the Galapagos medium ground finch (*Geospiza fortis*) in response to dryer conditions during an El Niño event (Boag and Grant 1981). Beak thickness increased, as birds with thicker beaks were better able to break into the harder, desiccated seeds, and consequently had higher fitness. The effect was later shown to be primarily due to a single locus (Abzhanov et al. 2004). At the same time, a 30 year study of the impact of selection on the medium ground finch and the cactus finch (*Geospiza scandens*) showed that the response was dynamic over that period, and that the end result after 30 years was unpredictable (Grant and Grant 2002). If the relevant trait can quickly evolve when environments change, as in this example, the population may not move, but may show signs of local contraction due to selective load. Tracking the evolution of functional genes under selection as environments change will be an important objective for the future as genomic data becomes more widely available. So far examples of this incorporating ancient DNA have mostly investigated disease resistance in human populations (e.g. Zawicki and Witas 2008).

A further possible response to changing environments is phenotypic plasticity, whereby behaviour or some plastic aspect of morphology or physiology changes to adapt to the new conditions (see Pigliucci 2001). Of course the capacity for a single genotype to have multiple phenotypic responses to environmental change will also have a genetic basis, and could evolve in response to a dynamic habitat. If the relevant traits are plastic when habitats change, population size and connectivity may not be affected, though this will also depend on the impact on other relevant species (e.g. prey, predator, competitor, etc.). Finally, a population will go extinct if it cannot move or adapt quickly enough, or survive the change as it is.

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Making predictions about how populations will respond to change is complex, and the key considerations may depend on factors that are not apparent just from current patterns of distribution and diversity. It is, therefore, useful to look back over past periods of environmental change and address questions about how populations were impacted then. By looking back over environmental change that happened over the course of the Pleistocene and Holocene (including climate change), we can track the impact on populations over those periods to gain insight into what may happen in the future. This will be the focus of this essay. Any general patterns that can be identified will have the potential to improve modeling of future impact, and in the face of climate change, this is becoming an important strategic focus for conservation effort, and therefore an important focus for conservation genetics.

Bottlenecks and founders

Many studies have investigated the impact of catastrophic environmental change and the consequent loss of diversity following population bottlenecks (see Garner et al. 2005 for a review). These studies are useful to help define the conditions that result in population crashes, and the relationship between the duration and severity of the crash, and the resultant loss of diversity. In some cases even quite small bottlenecks can preserve fairly considerable diversity, provided the demographic recovery is rapid (e.g. O'Brien et al. 2007), and small populations can retain diversity by chance (e.g. Lawrence et al. 2008). At the same time, even rapid recovery can generate strong differences between source and founder populations due to the distortion of allele frequencies by sampling effects during the bottleneck event. Lovett and Hoelzel (unpublished) compared two parallel bottlenecks from the same source population in Norway for reindeer (*Rangifer tarandus*). Each were well documented transfers of a small number of reindeer to whaling stations on South Georgia in the South Atlantic, either side of a glacier (ensuring no movement between the founder populations). The result was a relatively low loss of diversity despite the severe bottlenecks (seven and ten individuals), due to rapid demographic recovery, and a strong pattern of differentiation due to the sampling of alleles ($F_{ST} = 0.072$ comparing the two founder populations). While this situation provided a convenient case study, more typically the comparison of diversity before and after an historical bottleneck has depended on the extraction of DNA from ancient or historical samples (e.g. Groombridge et al. 2000; Hoelzel et al. 2002). Both direct comparative and simulation studies emphasize the high variance in outcome, and the potential for endpoints that differ substantially from

theoretical expectations (e.g. Hoelzel et al. 1993). Such information also facilitates predictions about how newly founded populations in emerging habitat may be impacted, or how severe local impact needs to be before significant diversity is likely to be lost. Further inference can be gained through tracking the loss of diversity over time (e.g. Chan et al. 2005). At the same time, it is important to understand the potential impact of environmental change on the connectivity and dynamics of larger populations.

Looking backwards through phylogeography

A well established literature, reviewed in Avise (2000) and extended since then, has applied phylogeographic methods to interpret the processes of population connectivity and dynamics over time. The phylogeographic approach has highlighted the role of the dominant Quaternary climate events, the ice ages together with the transitions into the warmer interglacial periods. Hewitt (2000) reviewed studies showing an impact on the distribution and population structure of a diversity of species in Europe. European species unable to cope with the colder climate or encroaching ice were apparently forced into southern refugia during glacial epochs, with re-emerging populations sometimes forming hybrid zones at points of secondary contact. Hewitt (2000) suggests a process of 'leading edge expansion' by which founder populations are established in suitable habitat. Founder events would lead to the loss of alleles and reduced heterozygosity. This partitioning of populations in refugia and consequent genetic drift could explain the phylogeographic signals reflected in the phylogenies of modern populations, and is broadly referred to as the 'expansion–contraction' model. Similar processes are proposed for populations in North America and elsewhere.

In a recent example Korstein et al. (2009) consider the expansion of brown bear (*Ursus arctos*) populations across Eurasia after the last glacial maximum. As for many of the earlier studies, they use mtDNA sequence data, and consider the geographic distribution of haplotypic lineages, in this case represented in median joining networks and Bayesian phylogenies. A common objective in these studies is to consider time points, interpreted from the phylogenies, in the context of known geologic events. This requires the assumption of a molecular clock, and therefore, an accurate interpretation of the mutation rate. However, although there are established estimates of mutation rate calculated by the phylogenetic method using geologic calibration points, more recent studies suggest that these estimates may be much too slow for intraspecific studies (see Ho et al. 2008; though considerable discussion continues in the literature on this topic).

Acknowledging the importance of applying an accurate rate, Korstein et al. (2009) incorporated sequence data from the cave bear (*Ursus spelaeus*) based on ancient DNA sequences (after Orlando et al. 2002). On this basis they derive a mutation rate within the brown bear lineages that is six times higher than the rate along the basal branch comparing the two species. The rate they calculated for brown bears, an order of magnitude higher than that typically used for the phylogeographic studies from a decade earlier, suggested an expansion in northern Eurasia following the most recent glacial maximum. The star-shaped phylogenies suggested population expansion, and a small (probably refugial) founder population. The close relationship between extant haplotypes further suggested a single Eurasian expansion event, and the geographically widespread occurrence of certain dominant haplotypes suggested no significant barrier to expansion. Comparing their data with published studies on various other mammalian species, they suggest that the brown bear model reflects a common theme for mammals in northern Eurasia. The conservation implication would be that remaining diversity should be maintained through the preservation of connectivity among geographic regions across the range.

In general, data from the Holocene timeframe will likely have greater relevance to our immediate concerns with conservation strategy in the face of anthropogenic impact. However there are exceptions. For example, a recent study investigated the phylogeography of species in Europe before the recent glacial maximum (Hofreiter et al. 2004). They sequenced ancient DNA from now extinct species (cave bear and cave hyenas; *Crocuta crocuta spelaea*) for samples ranging in age from 22000 to 72000 years old. While there was an indication of weak east vs west structure for the cave bear (as seen for post-glacial populations of a number of species, see Hewitt 2000), possibly reflecting a division created during the previous glacial epoch, there was no clear pattern for the cave hyena (though based on just 18 samples). The authors suggest that if continuity is the default state, disrupted by intermittent glacial epochs forcing populations into separate refugia, then interglacial periods should be characterized by migration gradually erasing the phylogeographic pattern established during the glacial maximum. They further suggest that conservation efforts should therefore be directed towards preserving and restoring connections between suitable habitats for species in temperate regions likely to have been affected by these cycles. However, it could be argued that founder events establishing refugial populations may affect the genetics of functional loci by drift or selection in a new environment, and that therefore migration between such populations may reduce fitness (cf. Hewitt 2000).

Historical demographics

Studies that focus on mtDNA phylogeography are limited in temporal resolution by the rate at which new diversity is accumulated by mutation, and by the resolution provided by extant genotypes. Furthermore, stochastic events and selection can affect the survival of lineages and the consequent information available in the tree. One way to improve this resolution is by including sequences of ancient DNA, especially in the context of the coalescent. The coalescent considers the structure of a phylogeny looking backwards in time to the points at which lineages come together (Kingman 1982), and has greatly facilitated studies investigating historical demography and connectivity. Including ancient DNA provides a more inclusive representation of coalescent events. Recent studies have used coalescent models, Bayesian statistics and Markov Chain Monte Carlo (MCMC) simulations to interpret population parameters based on both ancient and modern genetic data (e.g. Drummond et al. 2005). Modern computers are now powerful enough to search for the greatest likelihood posterior distributions in manageable analytical timeframes. At the same time, these methods are constantly being improved and refined (e.g. see Lopes 2010, this volume).

Bayesian coalescent methods have greatly facilitated our ability to look back at how environmental change impacted populations in the past, as they permit a relatively fine-scale assessment of population dynamics over time. Building on earlier similar approaches, Drummond et al. (2005) introduced the Bayesian skyline plot, and illustrated its potential by applying the method to published datasets. The Bayesian skyline plot uses MCMC simulations to estimate a posterior distribution of effective population size through time based on a sample of gene sequences and an appropriate nucleotide substitution model. Extending a study on the bison (*Bison antiquus*) that lived on the Beringian plains (between modern Russia and Canada) during the Pleistocene (Shapiro et al. 2004), Drummond et al. (2005) used this method to show that both climate (the last glacial maximum) and the arrival of humans coincide with periods of population decline in this species (see Fig. 1). The mutation rate applied was critical in matching these time points to the changes in population size, and this again was a rate that is more than an order of magnitude higher than those used in many earlier studies. It was calculated using a Bayesian MCMC approach that assesses intraspecific mutation rates through the comparison of sequences from known points in time (using ancient DNA and radiocarbon dating; Shapiro et al. 2004; Drummond and Rambaut 2007).

In an application of these methods to track more recent trends in population size, de Bruyn et al. (2009) report on a

Fig. 1 Study area and Bayesian skyline plot (BSP) for the bison of the Beringian plains (*left*; from Drummond et al. 2005), and the southern elephant seals from the Victoria Land Coast, Antarctica (after de Bruyn et al. 2009). *Dark lines* indicate BSP profiles (with the 95% highest posterior density intervals represented by shading), while the *grey line* in the background of the bison plot is an ordinary skyline plot (see Fig. 4 in Drummond et al. 2005 for further detail). Population size is given in units of effective female population size times the generation time (N_{ef}. T). *Source:* Bison photo by David Monnaiux; elephant seal and Ross Sea photos by Brenda Hall
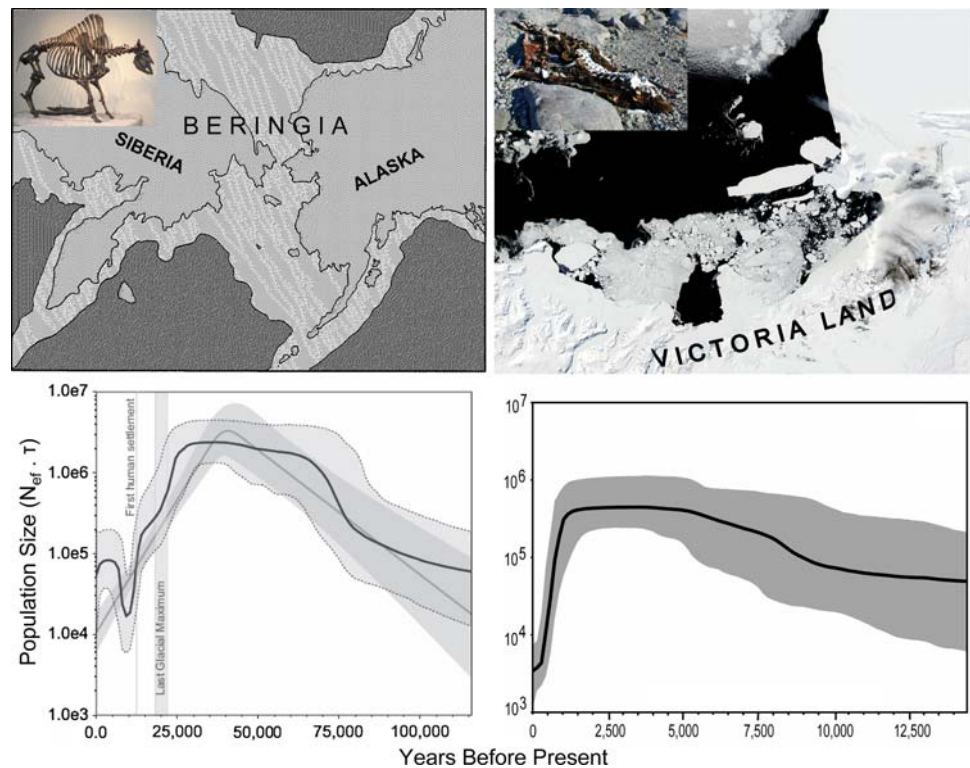
population of elephant seals at a breeding colony in the Antarctic. The sequencing of ancient mtDNA from more than 200 seals permitted the tracking of this population from its founding about 8000 years ago through to its extinction, 7000 years later (Fig. 1). In this case the mutation rate for the HVR1 segment of the mtDNA control region was calculated as 9.7×10^{-7} per site per year (using software described in Drummond and Rambaut 2007, and based on ancient DNA sequences from radiocarbon-dated samples ranging 8000 to 500 years old). This rate was consistent with various other rates derived for this locus based on intra-specific comparisons, including the mean human pedigree rate estimate derived from a meta-analysis (9.5×10^{-7} s.s.year^{-1}; Howell et al. 2003). Applying this rate, de Bruyn et al. (2009) found a very close match between the timing of relevant geologic events and elephant seal population dynamics. When ice retreated and new breeding habitat was exposed along the Victoria Land Coast in the Ross Sea, elephant seals quickly established a breeding colony there, which grew to over an order of magnitude larger than the source population on Macquarie Island. When the ice returned, the population crashed.

There were two important implications for conservation strategy. Southern elephant seals typically breed on sub-Antarctic islands that may be thousands of kilometers from where they feed in Antarctic waters. It is likely that their vagility taking them near where the new habitat was released is what permitted a rapid and successful response to change associated with the warming climate, and this my have relevance for other highly mobile species. At the same time, prey resource is abundant in Antarctic waters, so the established colony may not have been as vagile, and perhaps as a consequence, crashed when the Antarctic breeding habitat was lost. The other important implication is related to the fact that the established colony became very large, and likely significantly increased the number of elephant seals feeding in this region of the Antarctic Ocean. If similar habitat became available with warming temperatures in future, and again led to a substantial increase in the number of elephant seals feeding in that region, it would have knock-on effects on other species competing for the same resources.

Incorporating historical anthropogenic events

The previous few examples either require or are greatly facilitated by the use of ancient DNA. However coalescent methods can permit some strong inference based on modern molecular data alone. For example, Okello et al. (2008) studied an elephant (*Loxodonta africana*) population in northern Kenya using 20 polymorphic microsatellite DNA loci. The 400 animals biopsied represented about 40% of the total population, and their ages were also determined. Using a Baysian coalescent-based method (implemented in

the program MSVAR, Storz and Beaumont 2002) the authors found that for three out of four age cohorts, a detected decline in effective population size started about 2500 years ago during a period of climatic drying in tropical Africa. However, a third cohort representing the period 1971–1981 suggested a much more recent period of decline. Animals from this third cohort were sired during a period of intense poaching for ivory. In fact, in 1977 a survey counted more dead than live elephants (Poole et al. 1992). This intensity of poaching was short-lived, and the data suggest that diversity present prior to this period was carried through in surviving young as they matured and reproduced in later years. This study shows that the elephant population could recover because they are long-lived, and the strongest impact was comparatively brief, but that the result could have been "drastically different" if harvesting had continued for a full generation (Okello et al. 2008).

Another example, in this case driven primarily by anthropogenic impact, involves a study that combined investigation of ancient and modern DNA from New Zealand's endangered yellow-eyed penguin (*Megadyptes antipodes*). Boessenkool et al. (2009) discovered that the modern population represented a range expansion onto the New Zealand mainland South Island only within the last few hundred years. Although previously understood to be the declining remnant of a once abundant population, this study instead suggests a relatively recent founder population that replaced a now extinct and previously unrecognized sister species (*Megadyptes waitaha*). Modern populations on the South Island were represented in the same mtDNA lineage as for modern populations on Auckland and Campbell Islands, while the historical samples (dated approximately to AD 500–1700) were all represented in two separate lineages showing a genetic divergence from the modern populations of 2.2–4.2%. The authors suggest that the colonization of the mainland by the yellow-eyed penguin was permitted by the extinction of the native sister species, driven by human colonization and expansion into the South Island starting in the thirteenth century.

Conclusions

The theme of this essay has been on learning from the past to facilitate more effective conservation in the future. It seems clear that ancient DNA can play an important role in this, especially in the context of understanding how species have responded to past changes in climate, the gain or loss of habitat, and other pressures affecting their survival. Ancient DNA together with coalescent methods can improve estimates of historical demographic patterns, historical connectivity, splitting times among populations, directional migration (both in equilibrium and since the time of splitting;

e.g. Hey and Nielsen 2004), and crucially, substitution rates relevant to the time frame under consideration (see discussion in Ho et al. 2008). All other estimates related to the timing of historical events and effective population size based on genetic data depend on accurate substitution rates, and it is therefore encouraging that rates calculated in this way have allowed the derivation of very credible demographic patterns in the context of well known geologic time points (see examples discussed above). Given this window on the past, and methods that are improving all the time, we should be able to better incorporate details into models that try to predict future distributions, population size and patterns of connectivity, and further consider the potential impact on functional genes. The effectiveness of this approach will depend on how representative historical trends are, and the testing of these model predictions. However, as illustrated by many of the examples provided, we can already use inference gained from the past to identify true risks and natural processes, and this will facilitate the development of conservation strategy. In an increasingly complex and rapidly changing world, this type of improved insight will be essential to the design of lasting, effective conservation policy.

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