

INVITED REVIEW

Using phylochronology to reveal cryptic population histories: review and synthesis of 29 ancient DNA studies

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Abstract

The evolutionary history of a population involves changes in size, movements and selection pressures through time. Reconstruction of population history based on modern genetic data tends to be averaged over time or to be biased by generally reflecting only recent or extreme events, leaving many population historic processes undetected. Temporal genetic data present opportunities to reveal more complex population histories and provide important insights into what processes have influenced modern genetic diversity. Here we provide a synopsis of methods available for the analysis of ancient genetic data. We review 29 ancient DNA studies, summarizing the analytical methods and general conclusions for each study. Using the serial coalescent and a model-testing approach, we then re-analyse data from two species represented by these data sets in a common interpretive framework. Our analyses show that phylochronologic data can reveal more about population history than modern data alone, thus revealing 'cryptic' population processes, and enable us to determine whether simple or complex models best explain the data. Our re-analyses point to the need for novel methods that consider gene flow, multiple populations and population size in reconstruction of population history. We conclude that population genetic samples over large temporal and geographical scales, when analysed using more complex models and the serial coalescent, are critical to understand past population dynamics and provide important tools for reconstructing the evolutionary process.

Keywords: ancient DNA, bottleneck, gene flow, genetic diversity, genetic drift, genetic sampling, population structure, serial coalescent, temporal genetic data

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Introduction

Genetic data from modern populations are used frequently to infer demographic history, which includes changes in population size and the rates of movement between populations through time. Modern genetic data have proved invaluable in detecting population size changes due to human-induced decline or fragmentation of habitat in the recent past [e.g. elephant seals (Hoelzel *et al.* 1993), baboons (Storz *et al.* 2002), orangutans (Goossens *et al.* 2006)], and also in elucidating the population history of

relatively recent species like humans (Ramachandran *et al.* 2005). Inference of less recent demographic history is often more difficult because modern genetic data record the sum of all population processes through time, and are weighted strongly by the most recent and/or significant genetic events. Alternatively, if the demographic event happened a long time ago, the population could have reached genetic equilibrium by the time of modern genetic sampling, rendering the ancient demographic event undetectable. Additionally, several processes can result in similar patterns in the modern genetic data. For example, low levels of population subdivision between two populations could indicate either recent divergence or an older divergence followed by recent gene flow. Thus, true

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Box 1 Ancient DNA data: why are population genetic analyses difficult?

Issues	Solutions
Time-stamped data	Investigate sensitivity of events to timing
Noncontemporaneous sequences	Explicitly incorporate temporal factors
Error in age estimates, time-averaging	Incorporate errors in dating, use multiple dates
No sampling in certain time-intervals	Extrapolate ages between dates using clearly defined methods of reconstruction
Sample size	Investigate sensitivity of events to sampling
Limited/constrained sampling	Statistically investigate the impacts of lower sample size (e.g. using hypothesis testing)
Genetic data	Investigate sensitivity of events to mutation rates and selection
Reconstructing evolutionary history based on a single marker results in more error	New sequencing methods allow exploration of nuclear DNA
Nuclear data often with variable or unknown mutation rates and/or selection intensities	Standardizing with mitochondrial DNA useful
Possible errors in ancient sequences	See ancient DNA reviews described in text

demographic history can be difficult to reconstruct based on modern genetic data alone and even when the historic process is appropriately reconstructed, ascertaining the timing of such events is difficult.

Ancient genetic data from multiple populations and multiple points in time and their analyses allow a direct view of the past and can yield powerful insights into the timing and magnitude of events in the history of populations. Use of serially sampled genetic data permits a chronologic testing of the reconstructed population processes and is a particularly fruitful area for novel approaches in population genetics because such data allow us to ascertain the relative roles of microevolutionary processes through time. We term this approach phylochronology (Hadly *et al.* 2004) or the study of populations in space and time using phylogenetic and population genetic methods.

Ancient DNA studies and data have been used to answer questions regarding phylogenetic relationships between extant and extinct species (e.g. Shapiro *et al.* 2002), animal domestication (e.g. Leonard *et al.* 2002), human origins and anthropology (e.g. Adcock *et al.* 2001) and in rare cases, population genetics (e.g. Drummond *et al.* 2005; Valdiosera *et al.* 2008). Most reviews on ancient DNA focus on methodological problems relating to the retrieval of authentic genetic material (e.g. Hofreiter *et al.* 2001; Pääbo *et al.* 2004; Gilbert *et al.* 2005; Willerslev & Cooper 2005), or are a synopsis of the history and summary of studies in the field. Our approach here is somewhat different, stemming from a need for a synopsis of theoretical and analytical approaches to chronologic genetic sampling. Serial sampling decreases the stochastic variability of the coalescent processes and allows better parameter estimation (Drummond

et al. 2003). Although it is theoretically (Drummond *et al.* 2003), statistically (Ramakrishnan *et al.* 2005) and empirically (Chan *et al.* 2006) proven that ancient DNA data provide a better ability to investigate demographic history, the statistical analyses of ancient DNA data remain a challenge, especially in the case of phylochronologic data (Box 1).

First, we compiled a set of ancient genetic studies focused on the temporal analysis of populations within a species. We next reviewed the analyses of such population-level ancient DNA data sets and how these methods have been applied in published ancient DNA studies. Furthermore, we describe a novel, simulation-based approach for the analyses of ancient genetic data from populations and apply this approach to two exemplar ancient DNA data sets. Finally, we describe future directions in ancient genetic data and analyses.

Ancient genetic data from populations: current approaches to analyses

Analyzing patterns of genetic variation

While ancient genetic studies are relatively routine today (a SciSearch perusal showed 1155 aDNA papers published from 1990 to 2008, with one study in 1990, increasing to 138 by 2007), most do not cover ancient population genetics explicitly. We performed a literature search in current contents (242 records) and the Web of Science (288 records) under keyword 'ancient DNA' and 'population' to identify research that uses ancient DNA to investigate population level genetic variation. Based on the results of the searches, we summarize 29 of these studies in Table 1. Table 1 does

Table 1 Review of 29 ancient population genetic data sets

Study questions	Species	Location and age of samples	Genetic information	Analytical approach	Conclusions	Reference
Is diversity maintained between time intervals? Is there evidence of population subdivision?	Kangaroo rat (<i>Dipodomys panamintinus</i>)	49 museum specimens (< 100 yrs) and 63 modern specimens from three southern California populations (subspecies)	225 bp of mtDNA	Phylogenetic trees, summary statistics	No significant change in diversity or population structure through time.	Thomas <i>et al.</i> 1990
How does genetic variation in ancient rabbits compare to modern individuals?	European rabbit (<i>Oryctolagus cuniculus</i>)	110 specimens from archaeological sites ranging from 11 000 to 300 YBP distributed across France, Spain and Africa	90 out of 110 yielded 190 bp of the mtDNA cytochrome <i>b</i>	Haplotype networks, and comparison of haplotype frequencies across different regions	Data reveal stability of haplotypes through time.	Hardy <i>et al.</i> 1995
Are changes in phenotype during the late Holocene correlated with local genetic changes or driven by immigration?	Pocket gopher (<i>Thomomys talpoides</i>)	74 ancient specimens from one locality between 2400 YBP to present and 7 modern samples	164 bp of mtDNA	Haplotype-based inference	High structure in both past and present. Change in diastemal length due to plasticity in local population a response to climatic warming.	Hadly <i>et al.</i> 1998
Is the current phylogeographical pattern the same as the late Pleistocene pattern?	Brown bear (<i>Ursus arctos</i>)	9 ancient specimens ranging from 45 000 to 14 000 YBP; taken from several localities from northwestern North America	258 bp of the mtDNA <i>cyt b</i> and 132 bp of the mtDNA control region	Phylogenetic tree and haplotype-based inference	High phylogeographical structuring in the present. No structuring in the past. Change due to late Pleistocene events?	Leonard <i>et al.</i> 2000
Is current phylogeographical pattern reflected in the past in Beringia?	Brown bear (<i>Ursus arctos</i>)	30 ancient specimens from > 35 000 YBP and from 21 000 to 10 000 YBP	195 bp of the mtDNA control region	Phylogenetic trees and haplotype-based inference	Changes in haplotypes in a geographical region with time.	Barnes <i>et al.</i> 2002
What was population structure and genetic variability in the past?	Cave bear (extinct) (<i>Ursus spelaeus</i>)	29 ancient specimens from 80 000 to 12 000 YBP from caves across Europe	179 bp of the mtDNA control region	Phylogenetic trees, haplotype-based inference, population genetic summary statistics	Moderate levels of genetic variation in extinct bears. High gene flow and no population structure.	Orlando <i>et al.</i> 2002
What was population structure and genetic variability in the past?	Cave bear (extinct) (<i>Ursus spelaeus</i>)	12 ancient specimens from 49 000 to 26 500 YBP from 9 caves in Europe	282–286 bp of the mtDNA control region	Phylogenetic analysis, haplotype-based inference, simulations to compare diversity in brown bears vs. cave bears	Genetic diversity of cave bears lower than brown bears. Same haplotype found within a cave through time. High population structure.	Hofreiter <i>et al.</i> 2002

Table 1 Continued

Study questions	Species	Location and age of samples	Genetic information	Analytical approach	Conclusions	Reference
What is the mutation rate of the mitochondrial control region?	Adelie penguin (<i>Pygoscelis adeliae</i>)	96 ancient specimens from 6400 YBP to present and 380 modern individuals from Antarctica	390 bp of the mtDNA control region for samples > 2000 YBP; 663–1042 bp of the mtDNA control region from samples < 2000 YBP and 1600 bp of the mtDNA control region for modern samples	Median-joining networks, Bayesian MCMC approach based on the serial coalescent; gene flow not considered	Mutation rate 10 times higher than phylogenetic estimates suggest.	Lambert <i>et al.</i> 2002
How do ancient salmon relate ancestrally to modern populations?	Salmon (<i>Salmo salar</i>)	6 ancient specimens from 41 000 to 3250 YBP from Europe	Between 164 and 294 bp of the mtDNA ND1 region, polymerase chain reaction products subject to restriction fragment length polymorphism analyses	Haplotype-based analysis	Ancient salmon have a different haplotype than all modern samples.	Consuegra <i>et al.</i> 2002
Did genetic changes accompany domestication?	Maize (<i>Zea mays</i>)	11 ancient specimens between 4300 and to 900 YBP from North America	50–80 bp from <i>teosinte branched 1</i> , <i>prolamin box binding factor</i> , all three genes known to be important in cob nutritional quality	Haplotype-based analysis	Selection for both morphological and biochemically beneficial crop traits in maize happened as early as 4000 YBP.	Jaenicke-Despres <i>et al.</i> 2003
What caused the extinction of the Beringian bison?	Bison (<i>Bison bison</i>)	442 specimens ranging from 60 000 YBP to present from Alaska, North America and Eurasia	685 bp of the mtDNA control region	Bayesian serial coalescent approach used to investigate demographic model	Population decline started before human arrival	Shapiro <i>et al.</i> 2004
What were phylogeographical patterns like in the Pleistocene and how do they compare across four species?	Cave bear (extinct) (<i>Ursus spelaeus</i>) Brown bear (<i>Ursus arctos</i>) Neanderthal (<i>Homo sapiens neanderthalensis</i> , extinct) Cave hyena (<i>Crocota crocuta spelaea</i> , extinct)	40 cave bear specimens between 72 000 to 2000 YBP (continental Europe); 2 brown bears (47 420 and 39 940 YBP); 18 cave hyenas from 15 locations (age ranged from 50 000 to 37 000 YBP); published sequences from 4 Neanderthal specimens from 42 000 to 29 000 YBP from Germany, Russia and Croatia.	Cave bears amplified for 134 bp of the mtDNA control region; brown bears amplified for 270 bp of the mtDNA control region; Cave hyenas amplified for 366 bp of the mtDNA cytochrome <i>b</i> ; Neanderthals amplified for 333–337 bp of the mtDNA control region	Phylogenetic inference and haplotype networks	None of the four species reveal phylogeographical patterns. Since only one of the four species has a modern phylogeographical pattern, these data question the stability of phylogeographical patterns through time.	Hofreiter <i>et al.</i> 2004a

Table 1 Continued

Study questions	Species	Location and age of samples	Genetic information	Analytical approach	Conclusions	Reference
Was there gene flow between morphologically distinct populations of cave bears in Europe?	Cave bear (<i>Ursus spelaeus</i>)	Specimens were collected from 3 caves: 2 are 10 km apart but at different altitudes, and 1 is 200 km away; 9, 7 and 10 samples were from 50 000 to 20 000 YBP from each of the 3 caves.	All samples sequenced for 285 bp of the mtDNA control region	Haplotype-based inference; morphological measurements of metapodial bones; Ewens-Watson type sampling calculations to investigate the possibility of observing differences between 2 close caves by chance.	No gene flow between higher altitude and lower altitude caves, even though they are very close. Evidence for gene flow between lower elevation caves.	Hofreiter <i>et al.</i> 2004b
Do gophers and voles respond similarly to changes in the climate?	Montane vole (<i>Microtus montanus</i>) Pocket gopher (<i>Thomomys talipodes</i>)	47 ancient specimens from 3000 YBP to present and 48 modern samples for montane voles 76 ancient specimens from 3000 YBP to present	All vole samples amplified for 312 bp of the mtDNA cytochrome <i>b</i> ; pocket gopher mtDNA sequences were 64 bp	Hypothesis-testing approach based on coalescent simulations. Ewens-Watson tests to investigate effects of sampling.	Analyses revealed that changes in genetic diversity observed across time for voles are not consistent with a closed population. Results robust to possible demographic fluctuations as well as assumptions regarding mutation rate.	Hadly <i>et al.</i> 2004
The musk oxen has experienced a severe range contraction and is now restricted to a very small part of its habitat. Was this accompanied by a decline in genetic diversity?	Musk ox (<i>Ovibos moschatus</i>)	9 specimens from 45 000 to 700 YBP from across the Holarctic	All samples amplified for between 114–374 bp of both the mtDNA control region and cytochrome <i>b</i>	Phylogenetics, phylogeography and population genetic summary statistics	Higher genetic variation in the samples from the past.	MacPhee <i>et al.</i> 2005
Do microsatellite allele frequencies change over time?	Adelie penguin (<i>Pygoscelis adeliae</i>)	15 ancient specimens from a single time horizon at 6000 YBP and 48 modern samples	All samples genotyped at 9 microsatellite loci	Histograms and pie charts investigating change in allele frequency	Analyses revealed that allele frequencies change over time.	Shepherd <i>et al.</i> 2005

Table 1 Continued

Study questions	Species	Location and age of samples	Genetic information	Analytical approach	Conclusions	Reference
Did ancient populations of tuco-tuco have greater genetic variability than exists today? Can we quantify the magnitude and timing of the bottleneck?	Tuco-tuco (<i>Ctenomys sociabilis</i>)	14 specimens from 850 to 150 YBP from one cave and 33 samples between 10 000 to 3300 YBP from another, proximate cave	All samples amplified for 253 bp of the mtDNA cytochrome <i>b</i>	Approximate Bayesian computation or rejection algorithm approach implemented through the serial coalescent, population genetic summary statistics. Also investigated sensitivity to various model assumptions.	Posterior distributions for demographic parameters reveal a strong signature for a population bottleneck around 1600 generations ago. Pre-bottleneck N_e was around 53 000 and post bottleneck N_e was around 1000.	Chan <i>et al.</i> 2006
Do species track their habitat during glacial cycles and associated habitat shrinkage, or do populations outside refugia go extinct?	Arctic fox (<i>Vulpus lagopus</i>)	7 specimens across Europe	All samples amplified for 292 bp of the mtDNA control region	Haplotype networks	No evidence for habitat tracking.	Dalen <i>et al.</i> 2007
Can haplotypes be replaced over time?	Cave bear (<i>Ursus spelaeus</i>)	29 specimens from Southern Germany ranging from 38 000 to 25 000 YBP	20 samples were amplified for 134 bp of mtDNA control region	Haplotype networks, randomization tests to investigate whether haplotype-temporal correlation can be observed by chance	Low probability that such a change could be observed by chance. Authors infer haplotype replacement.	Hofreiter <i>et al.</i> 2007
How has the genetic variability in the Svalbard stock of bowhead whales changed through the Holocene and how different were the ancient Svalbard whales from modern bowheads of other genetic stocks?	Bowhead whale (<i>Balaena mysticetus</i>)	105 ancient specimens ranging from older than radiocarbon to late Holocene (< 3000 YBP)	99 samples were amplified for 453 bp of the mtDNA control region	Phylogenetic analyses and population genetics based summary statistics	No significant loss of diversity through time (in spite of historical population bottleneck) and no evidence for population structure between the North Pacific (modern samples) and the North Atlantic (ancient samples).	Borge <i>et al.</i> 2007

Table 1 Continued

Study questions	Species	Location and age of samples	Genetic information	Analytical approach	Conclusions	Reference
Were brown bears confined to glacial refugia during glacial periods in late Quaternary Europe?	Brown bear (<i>Ursus arctos</i>)	66 specimens from Spain, Southern France, Germany, Italy and Romania. Specimens range between 17 440 YBP to 1570 YBP.	21 ancient samples yielded 246 bp of mtDNA control region, 10 modern samples	Phylogenetic analyses, haplotype networks and population genetic summary statistics.	No population genetic structure observed as would be expected if the populations were confined to glacial refugia.	Valdiosera <i>et al.</i> 2007
Did the mammoth population in Beringia experience change in population size before the Last Glacial Maximum?	Woolly mammoth (extinct) (<i>Mammuthus primigenius</i>)	96 specimens from across Beringia, dating from > 60 000 to 12 000 YBP	41 samples yielded 741 bp of partial sequence from the mtDNA for cytochrome <i>b</i> , proline tRNA and control region	Phylogenetic analyses and molecular clock analyses to investigate timing of clade expansion, skyline plots	Range expansion of mammoths from Eastern to Western Beringia.	Barnes <i>et al.</i> 2007
Was the Beringian wolf different (ecologically and genetically) from the modern Alaskan gray wolf?	Gray wolf (<i>Canis lupus</i>)	20 specimens from Eastern Beringia ranging from > 60 000 to 12 600 YBP	All samples amplified for 425 bp of mtDNA control region	Phylogenetic inference and population genetic summary statistics	Modern genetic diversity is decreased compared to Pleistocene. Morphometric and isotopic analyses reveal that the Pleistocene gray wolves were a unique ecomorph that is now extinct.	Leonard <i>et al.</i> 2007
How has genetic variation in three species of brown kiwis changed over the last 30 000 years?	North Island brown kiwi (<i>Apteryx mantelli</i>), rowi (<i>Apteryx rowi</i>) and tokoeka (<i>Apteryx australis</i>)	46 ancient specimens between 4000 and < 2000 YBP; 58 modern brown kiwi	28 samples yielded 641 bp of mtDNA cytochrome <i>b</i> and 200 bp of mtDNA control region	Phylogenetic analyses, population genetic summary statistics and nested clade analyses	Phylogenetic trees revealed that including ancient samples did not change the species definitions. Population genetic summary statistics revealed that all species had lost genetic variation.	Shepherd & Lambert 2008

Table 1 Continued

Study questions	Species	Location and age of samples	Genetic information	Analytical approach	Conclusions	Reference
Was the Iberian peninsula a glacial refugium and a source for recolonization of European brown bear populations? What is the demographic history of Iberian brown bears?	Brown bear (<i>Ursus arctos</i>)	13 specimens from the late Pleistocene and Holocene from Iberia	117 bp (9 samples) and 115 bp (4 samples) of mtDNA control region. 177 bp from 24 modern samples.	Phylogenetic inference and population genetic summary statistics. Time windows used (Pleistocene, Holocene and present). Serial coalescent simulations used to investigate whether observed change in gene diversity is consistent with constant population size.	The demographic history of the Iberian brown bear does not reflect a constant population size. The data reveal evidence of both a recent decline in gene diversity, but also many older bottlenecks.	Valdiosera <i>et al.</i> 2008
Were the North African brown bears genetically distinct from other brown bears?	Brown bear (<i>Ursus arctos</i>)	7 specimens spanning the Pleistocene-Holocene transition (from 9620 to 1285 yBP) from Algeria and Morocco	All samples were amplified for 269 bp of mtDNA control region, modern brown bear sequences were included	Phylogenetic analyses and genetic distance between brown bear clades	North African brown bears comprised a unique and divergent mtDNA clade that went extinct relatively recently. Authors highlight the importance of the Pleistocene-Holocene transition as an important climatic event compared to the last glacial maximum.	Calvignac <i>et al.</i> 2008
Were pre-harvest sea otters in Oregon genetically closer to the northern or the southern subspecies?	Sea otter (<i>Enhydra lutris</i>)	16 specimens between 2000 and 170 yBP from archeological sites in Oregon, USA	All samples were amplified for 222 bp of mt DNA control region	Haplotype networks, haplotype comparisons and population genetic distance between clades	Pre-harvest sea otters in this region have closer genetic affinities to otters from the southern versus northern subspecies.	Valentine <i>et al.</i> 2008

Table 1 *Continued*

Study questions	Species	Location and age of samples	Genetic information	Analytical approach	Conclusions	Reference
Does whole mitochondrial DNA sequence using high throughput sequencing provide additional insight into mammoth phylogeography?	Woolly mammoth (extinct) (<i>Mammuthus primigenius</i>)	5 specimens ranging between older than radiocarbon (> 60 000 YBP) and 1399 YBP	Complete mitochondrial genome for all samples	Phylogenetic analyses based on complete mitochondrial genomes, stochastic simulations to investigate clade extinction and functional analyses of mtDNA proteins to investigate role of selection.	Phylogenetic analyses reveal ancient divergence between clades. Protein function analyses reveals that sequence changes observed in new clade need not have impaired or enhanced functionality. Authors conclude that the clade might have been lost due to drift.	Gilbert <i>et al.</i> 2008
What was the genetic relationship between North American and Asian mammoths?	Woolly mammoth (extinct) (<i>Mammuthus primigenius</i>)	108 specimens spanning radiocarbon time, with the youngest sample at 4000 YBP. Analyses included 52 previously published sequences.	All samples were amplified for 743 bp of the hypervariable region of mtDNA	Bayesian analyses of the Stirling probability of theoretically expected haplotype richness; rooted reticulated tree using Bayesian and network methods to discern haplogroups and their spatial distribution; BEAST and relaxed molecular-clock analyses to infer timing of haplotype divergence; Bayesian skyline plots to investigate demographic history of Asian and North American mammoths.	Ancient origins of mammoths in Asia, followed by their expansion into eastern Beringia (North America) was followed by movements of North American mammoths into Asia about 300 000 years ago. This movement resulted in a potential replacement of Asian mammoths, suggesting that before extinction, mammoth genetic diversity was characterized by an 'out of America' event. Mammoths show similar demographic trends (older expansion and more recent decline) as bison in the same region, although the timing of these events is different.	Debruyne <i>et al.</i> 2008

YBP = years before present.

not include studies on human populations, those based on archaeological samples or museum specimens (up to 1500 years old), studies of domesticated species or research that includes less than three ancient samples. All studies summarized in Table 1 have used phylogenetic and phylogeographical methods to infer population history. In populations with simple histories or affected by particular environmental events such as immigration (e.g. Barnes *et al.* 2002), or overhunting (e.g. Weber *et al.* 2000), this type of an approach can be straightforward and revealing. However, the biggest drawback is that the temporal aspect of sampling is not considered explicitly. Furthermore, this type of an inferential framework does not statistically address alternative hypotheses, which has been an important part of phylogeographical studies with modern data (Knowles & Maddison 2002). For example, we might observe haplotype turnover at a location over time, and in a temporally implicit framework, infer this to be caused either by changes in population size, migration rate or mutation rate. However, haplotype turnover through time is also possible due simply to genetic drift. Furthermore, describing patterns of genetic variation based on haplotype networks and/or summary statistics alone does not allow us to test multiple hypotheses, quantify the parameters involved in the reconstruction of population history, or to investigate the sensitivity of our conclusions to the parameters chosen. An example of the limitations of such methods is provided by the almost contemporary studies of European cave bears (Hofreiter *et al.* 2002; Orlando *et al.* 2002), where researchers inferred very different population histories from similar data sets. Thus, although descriptions of ancient genetic data based on summary statistics and/or haplotype networks are invaluable, they are somewhat restrictive and may have alternative, or multiple, processes shaping them. True reconstructions of population history require more substantive analyses.

Parameter estimation. Beyond description of ancient genetic data, we aim to reconstruct population history in order to better understand the evolutionary process. We are interested in estimating the species effective population size and deriving a mutational model, and possibly even rates of migration between populations. The coalescent (Kingman 1982; Hudson 1990) is a modelling framework that has been used extensively in population genetics: both as an analysis tool to understand modern data as well as an exploratory tool to investigate the possible impact of complex evolutionary histories. Similar to using the coalescent, the serial coalescent (Rodrigo & Felsenstein 1999) can be used to model ancient genetic data. As the name suggests, the serial coalescent models samples through time. This allows us to treat both modern and ancient samples as part of the same evolutionary process and promises to hold deeper insight into the evolutionary dynamics within populations.

Parameter estimation in the context of ancient genetic data (Table 1) is based on the serial coalescent, implemented in both a fully Bayesian (e.g. Drummond *et al.* 2005) and an approximate Bayesian framework (e.g. Chan *et al.* 2006).

Parameter estimation using Markov Chain Monte Carlo. Markov Chain Monte Carlo (MCMC) methods recently have gained popularity in studies of population history (Beaumont & Rannala 2004). MCMC methods also have been applied to ancient genetic data (Table 1), especially using the more recent versions of the program BEAST (Drummond & Rambaut 2007), where these methods are implemented. The MCMC framework implemented in BEAST has allowed estimation of parameters including mutation rate in penguins (Lambert *et al.* 2002) and changes in population size associated with Pleistocene glaciations and first human contact for bison (Shapiro *et al.* 2004). Although Bayesian MCMC provides flexible methods to model mutational processes by incorporating various models of molecular evolution, it is less flexible with regard to demographic history. Current implementations in BEAST allow a single population of constant size, exponential and/or logistic growth. The MCMC models are not capable of modelling data from multiple populations because the likelihood functions are only defined in the case of single population models.

Parameter estimation using Approximate Bayesian Computation. Parameter estimation in a purely Bayesian or likelihood-based framework requires a likelihood function, which is not defined except in single population historic scenarios. Although likelihood functions cannot be defined for coalescent models including more than one population, we can use simulations of population history as a tool for parameter estimation. Computationally intensive methods such as rejection algorithms (Fu & Li 1997) and more recently, Approximate Bayesian Computation (ABC, Beaumont *et al.* 2002) recently have been applied in the context of ancient DNA data sets. The rejection algorithm estimates posterior distributions for a set of population genetic parameters by comparing observed summary statistics to simulated values for a range of demographic histories, while retaining compatible histories. Initial implementations of the rejection algorithm used standard rejection criteria. Recent statistical advances of the rejection algorithm, or ABC, use enhanced methods for sampling the prior parameter space (Marjoram *et al.* 2003) and rejection criteria (Beaumont *et al.* 2002).

The rejection algorithm is implemented (as in Beaumont *et al.* 2002) in the program Serial SimCoal (Anderson *et al.* 2005; <http://www.stanford.edu/group/hadlylab/ssc/BayeSSC.htm>), and has been used to investigate the intensity and timing of the population bottleneck experienced by *Ctenomys sociabilis* (Chan *et al.* 2006) and population

size and migration of Iberian brown bears (Valdiosera *et al.* 2008).

Bayesian and regular skyline plots

Skyline plots were developed in response to the simplistic parameter estimation frameworks (single population, constant size or exponential growth) provided by the coalescent. Sequence alignment can be used to reconstruct the rate of coalescence through time, or a lineage through time plot (Nee *et al.* 1995). This can be transformed into estimates of effective size through time (Pybus *et al.* 2000) or the classic skyline plot. This version of the classic plot assumes high phylogenetic signal and a well-resolved phylogeny. Since this is often not the case with ancient genetic data from and between populations of a species, this approach was extended into the generalized skyline plot (Strimmer & Pybus 2001). The generalized skyline plot allows multiple coalescent events to be grouped together, and hence allows inclusion of data sets that include identical sequences. Additionally, the generalized skyline plot allows calculation of coalescent error so it can be used to compare various demographic models. The generalized skyline plot is not computationally intensive, and hence, can be used very effectively as a tool to explore various demographic historic scenarios. However, it requires assumption of a basal phylogenetic tree and hence a set of divergence times, excluding evolutionary stochasticity from the process of estimating demographic history. This shortcoming of the skyline plot was addressed by Drummond *et al.* (2005) in the Bayesian skyline plot, which uses MCMC to estimate a posterior distribution for effective population size through time and allows for phylogenetic and coalescent uncertainty. The Bayesian skyline plot was applied to one of the largest ancient DNA data sets, that from the Beringian bison (Shapiro *et al.* 2004). Earlier analyses of these data with a generalized skyline plot revealed a population decline starting at 30 000–40 000 YBP. However, the Bayesian skyline plot discerns a secondary decline at 10 000 YBP, followed by a recent population size recovery. This second bottleneck corresponds roughly the timing of human settlement of this region. Thus, the Bayesian skyline plot allows the exploration of more complex demographic scenarios. However, it is still limited to a single population context, and does not address the analyses of phylochronologic data.

Hypothesis testing using coalescent simulations

With the ability to model temporal genetic data comes the powerful ability to use simulations to test whether observed data fit within proposed hypotheses. The greatest advantage of this approach is the ability to parameterize biologically plausible population histories, and then investigate their

feasibility given the observed data. A major disadvantage of hypothesis testing is that although it allows you to reject certain hypotheses or historic scenarios, it does not necessarily reveal the true population history. Additionally, it does not help to distinguish between several plausible population histories.

These shortcomings of hypothesis testing are evident in ancient DNA studies where it has been applied. Hadly *et al.* (2004) investigated ancient genetic data from before and after the Medieval Warm Period (a global warming event approximately 1000 years ago) for gophers and voles from Lamar Cave in Yellowstone National Park, Wyoming, USA. Fossils from this cave revealed a decline in abundance for both gophers and voles. However, analysis of the ancient DNA of vole specimens showed an associated increase in genetic diversity. Hadly *et al.* (2004) used coalescent simulations to conclude that the Lamar Cave vole population was not closed (implying immigration). They did so by showing that the observed change in genetic diversity was not possible based on changes in population size or mutation rate alone. Strictly speaking, the hypothesis testing in this study ruled out a closed, single population model. Belle *et al.* (2006) and Valdiosera *et al.* (2008) also implemented simulation-based hypothesis testing. Analyses by Belle *et al.* (2006) rejected the claim that modern Tuscans are direct descendants of ancient Etruscans, but the study could not discriminate between alternative gene flow scenarios between the Etruscans and the Tuscans. Valdiosera *et al.* (2008) used the simulation approach to ascertain that the Iberian brown bear was not previously genetically isolated, in stark contrast to the population's isolation of today.

Ancient population genetics: statistical and biological inferences so far

Over the past 18 years, studies involving ancient DNA have focused primarily on phylogenetic and phylogeographical inference, with an additional emphasis on demographic history. Novel molecular biology methods have allowed the size of data sets to increase steadily (e.g. Gilbert *et al.* 2008). Additionally, more recent studies use a variety of analysis methods to address questions with the data (e.g. Debruyne *et al.* 2008). In some cases, increased sampling and additional analyses have even resulted in a revised interpretation of demographic history (e.g. mammoths: Gilbert *et al.* 2008 and Debruyne *et al.* 2008). More recently, ancient DNA studies are being used to shed light on conservation issues in extant endangered species (e.g. Borge *et al.* 2007; Shepherd & Lambert 2008). But what have ancient DNA studies taught us so far about population processes?

Many of the ancient DNA studies summarized in Table 1 detect changes in population size. In terms of timescale,

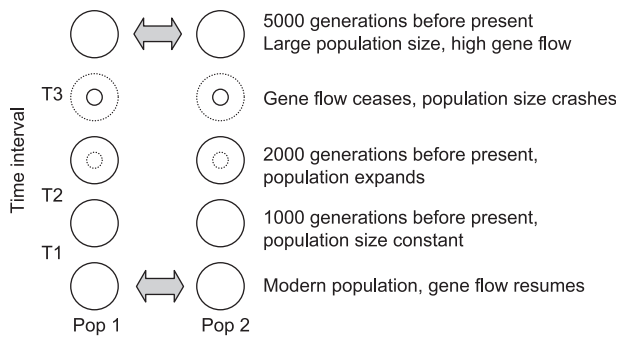


Fig. 1 Two hypothetical populations illustrated with 5000 generations of population history, including changes in population size (size of circle) and gene flow (presence and thickness of arrows). Sampling multiple populations through time would allow us to reconstruct population history. Use of a 'time window' allows us to investigate population history during these times. The temporal sampling windows shown are from modern to 1000 generations ago, from 1000 to 2000 generations ago and from 2000 to 5000 generations ago.

most studies detect population size decline in the last few thousands of years, induced by humans. However, some studies reveal population decline before human impact, often coincident with environmental changes (Shapiro *et al.* 2004; Chan *et al.* 2006; Leonard *et al.* 2007; Debruyne *et al.* 2008). These results are important for population genetic theory, because they confirm that populations do not have constant sizes through time. This contradicts assumptions made by the majority of population genetic theory and its implementation and has implications for the investigation of population demographic history from modern genetic data alone.

A much lower proportion of the studies in Table 1 investigate changes in migration patterns through time. The brown bear data (Leonard *et al.* 2000; Barnes *et al.* 2002; Valdiosera *et al.* 2007; Valdiosera *et al.* 2008) exemplify such a study and reveal that the population structure observed in modern brown bears is relatively recent, and Hadly *et al.* (2004) use hypothesis-testing to rule out a closed population following a climatic event in the Holocene for montane voles.

The ability to investigate ancient population structure is fascinating, but severely restricted by sampling, which is opportunistic in ancient DNA studies. Given the low local abundance of large-bodied species like bison and bears, the chances of extensive ancient DNA samples for such species from multiple spatial locations is low. It is possible that small mammal systems (like rodents) because of their high local abundance might be ideal for sampling intensive studies of population structure through time (Tougaard & Renvoisé 2008).

Detecting changes in population structure or changes in population size are inherently descriptive of demographic

history, but do not necessarily address the mechanisms that drive evolutionary change in species through time. Few ancient genetic studies try to investigate species response to climatic change using ancient genetic data, where they correlate the observed changes in genetic diversity (Hadly *et al.* 2004; Chan *et al.* 2006; Dalen *et al.* 2007) and morphology (Hadly *et al.* 1998; Leonard *et al.* 2007) with a timed climatic event. Alternatively, comparative phylogeography based on ancient genetic data from different species in the same region has been used to elucidate the biological/ecological factors that drive species' genetic variation (Hofreiter *et al.* 2004a; Riddle *et al.* 2008). Such studies allow results from ancient genetic data to feed into our understanding of population genetic variation in the context of species' ecology, and are particularly insightful for ascertaining how environmental change fuels genetic change within species.

Model-based approaches to understanding the past

Figure 1 shows a schematic representation of the hypothetical history of two populations. Modern genetic data from these populations may not properly reconstruct the true population history, meaning that most population historic events remain 'cryptic'. The genetic signature of these cryptic processes are either lost (if the populations regain equilibrium following the change in size or gene flow before modern sampling), accounted for by incorrect processes (i.e. gene flow vs. population size increase) or averaged across multiple population processes (e.g. although there is no gene flow between 5000 and 1000 generations ago, we could still predict an average rate of gene flow based on the modern data alone). Using this example, we introduce the 'temporal sampling window', a practical approach that allows us to bracket particular demographic events in the history of populations using genetic information only from that particular time span. In the case of our Fig. 1 example, genetic samples from modern populations and from populations 1000, 2000 and 5000 generations in the past permit us to better reconstruct population history. We can do so using three time intervals: (i) between modern and 1000 generations in the past, (ii) between 1000 and 2000 generations in the past, and (iii) between 2000 and 5000 generations in the past. Given genetic data from different times in the past and additional data from populations in different geographical locations, we can then use an approach that uses the serial coalescent applied to several temporal sampling intervals to reveal population historic processes. In our analysis framework, we divide the past into discrete time intervals, empirically derived from radiocarbon dating of ancient genetic specimens indexed against generation time of the particular species. We then aim to reconstruct history for these populations during each of these temporal intervals,

ultimately placing them in the context of other the temporal intervals. We contend that this approach, an example of an analysis framework within phylochronology, when applied to data from both spatial and temporal specimens, is essential for reconstructing more complex patterns of population history and revealing cryptic processes. Furthermore, and perhaps most importantly, this approach very clearly defines the criteria (population size, migration rate, generation time, radiocarbon age, mutation rate, etc.) used for each case study, without which comparisons between studies, populations, and species is not possible. We explain the practical aspects of how this approach is implemented in Box 2.

One potential confounding factor in many ancient population genetic studies is that temporal samples are not necessarily from the same locality. Temporal samples from non-overlapping spatial localities are likely to confound apparent temporal variation (and potential microevolution) with standing geographical variation between populations. The complication of confusing within- vs. between-population genetic diversity is intensified in species with high F_{ST} values and in metapopulations, but use of our approach helps to illuminate whether high spatial variation might explain discrepancies in the data and whether it

confounded the correct interpretation of history. Thus, another outcome of our approach is a guide on whether increased spatial or temporal sampling may help to discriminate between alternative scenarios of population history.

In the following sections, we demonstrate how our approach can be applied to ancient genetic data sets using two species as examples (penguins and brown bears). We use serial coalescent simulations (Anderson *et al.* 2005) of data from specified temporal intervals to put these data sets into a common analytical framework. We choose these two case studies because (i) they are both relatively simple and allow us to explain our approach; (ii) the penguin data are of microsatellites, for which there is currently no analysis framework; and (iii) the brown bear data provide an opportunity to synthesize over multiple studies and specifically showcase the importance of considering population structure.

Case study 1: ancient genetic data from penguins

Shepherd *et al.* (2005) compared ancient penguin specimens in the Antarctic to modern individuals from the same locality. Given the excellent preservation of these samples,

Box 2: How to use a serial coalescent model-testing framework

- 1 *Assign temporal and spatial sampling windows.* First, bin the data into temporal intervals and spatial bins, or populations. This grouping might be based on specific temporal or geographical hypotheses researchers intend to test, permitting explicit investigation of a known geological or climatic event on population processes (e.g. Shapiro *et al.* 2004). For example, Hadly *et al.* (2004) were interested in the climatic impact of the Medieval Warm Period and grouped samples so that they could assess population processes before, during and after the event.
- 2 *Calculate summary statistics.* Next, calculate inter- and intrapopulation summary statistics for the binned data. For mitochondrial DNA we recommend average pairwise difference and number of segregating sites for the intrapopulation statistics because these two statistics reveal different aspects of population history. For interpopulation statistics, we use F_{ST} , which can illustrate important features of both spatial and temporal populations. For microsatellite data, we recommend calculation of heterozygosity and allelic variance for intrapopulation statistics and $(\delta\mu)^2$ for interpopulation statistics.

- 3 *Explore simple models.* We use a simple model to determine whether a single population, constant-size model is sufficient to explain the observed data. For example, we began our exploration by investigating the N_e parameter space using a logarithmic scale. That is, we investigated population sizes of 1000, 2000, 5000, 10 000, 20 000, 50 000, 100 000, 200 000, 500 000 and 1 000 000 and so on. We used Serial Simcoal (www.stanford.edu/group/hadlylab/ssc/BayeSSC.htm) and set up input files according to the specified sampling windows and the effective sizes, depending on the data and hypotheses we were using. Simulations were run 5000 times at each of these population sizes. Likelihoods of the observed data were then calculated as in Belle *et al.* (2006) and we tabulated individual statistics and cumulative likelihoods over the range of effective population sizes (see case studies). Next, we ensured that the parameter space has been thoroughly explored (e.g. do the likelihoods generated span a range of high to low, for the range of N_e investigated?). If they do not, it is important to use an iterative approach to increase the parameter space sampled. Next, contrast the likelihoods in the temporal and spatial windows. Do all temporal windows have similar effective population sizes? Do the interpopulation statistics

Box 2 *Continued*

fit single-population models? Qualitative answers to such questions allow us to develop the next set of more complex models if the simple models are not well supported. An example of this process is explained in the brown bear case study herein.

4 Investigate increasingly complex models. In the next stage, we investigated more complex models of population history. In our case, they included single-population models with changes in population size through time (as in the penguin case study) or two (or more) population models (as with brown bears). Again, any new parameter that is introduced is investigated at a logarithmic scale. If the simple model analysis demonstrates high likelihoods for particular effective population sizes, we reduce the effective size parameter space for complex models. In our case, we did so, since the earlier analyses defined the parameter space explaining the observed intrapopulation genetic diversity.

5 Test the models and perform sensitivity analyses. After exploring simple and more complex models, we tabulated the maximal likelihoods and the number of parameters for each of our models. We then calculated the AIC value (the value of the likelihood penalized by the number of parameters, as specified by Burnham & Anderson 2002) for each model. The model with the lowest AIC value provides the best statistical fit. ΔAIC values ($AIC_{\text{model}} - AIC_{\text{lowest}}$) reveal whether various models provide equivalent or statistically worse fit for the observed data (using criteria in Burnham & Anderson 2002). It is also possible to use additional simulations to evaluate how sensitive our results are to mutation rate (both absolute value and rate heterogeneity, range constraints for microsatellites), sample size and various other data uncertainties such as the age of the sample or timing of the event (as in Chan *et al.* 2006).

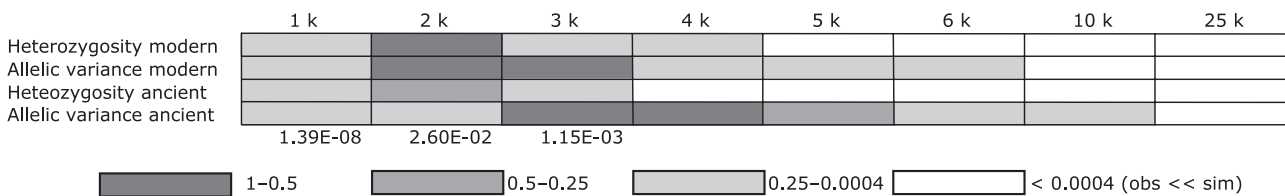


Fig. 2 Probabilities (binned into four categories with darker coloured cells representing higher probability) for four different summary statistics for the penguin ancient genetic data as a function of effective size for a constant-sized model. The overall likelihoods are presented below each effective size column.

the authors were able to genotype nine microsatellite loci from 15 ancient samples and 45 modern samples. All ancient samples were from one time horizon of around 6000 YBP. The authors used intrapopulation summary statistics calculated for both the ancient and modern samples to infer that the genetic composition of the population had changed. They inferred that this change was potentially due to higher population structure in past populations compared to those in present times.

We analysed these data using our temporal interval approach following Box 2:

- 1 Assign temporal and spatial sampling windows.** In the case of this data set, we only have two temporal windows [ancient (6000 YBP) and modern] for a single population since all the samples are from the same location.
- 2 Calculate summary statistics.** We calculated heterozygosity (0.657, 0.615) and allelic variance (2.267, 3.533) for both the modern and ancient data, respectively. All statistics were averaged over all the nine loci.

3 Explore simple models. We first explored the effective population size parameter space for a single population model. Because the data set included nine microsatellites, the simulations were run 45 000 times (9×5000) and the simulated statistics were also averaged across loci to get a total of 5000 simulated values. All four statistics (heterozygosity and allelic variation for both modern and 6000 YBP) were used to calculate the likelihood of each effective population size. Figure 2 reveals concordance in the statistics: models with effective size ≥ 5000 and ≤ 1000 do not fit the data, and that the data best fit an effective size of 2000. However, notice that the allelic variance is higher in the ancient genetic data, suggesting that the effective population size was higher in the ancient population. We use this inference as a guide to set up more complex models of demographic history.

4 Investigate increasingly complex models. Using the results from above, we chose models which include population decline in penguins and investigate whether such models fit the data better. Figure 3 reveals that although a

Table 2 Model testing using AIC for the penguin ancient and modern genetic data

Model	Likelihood	Parameters	AIC	Δ AIC
Constant size	0.026	1	5.171	
Population declines between ancient and modern	0.0275	2	7.121	1.95
Population increases between ancient and modern	4.66×10^{-12}	2	26.663	21.49

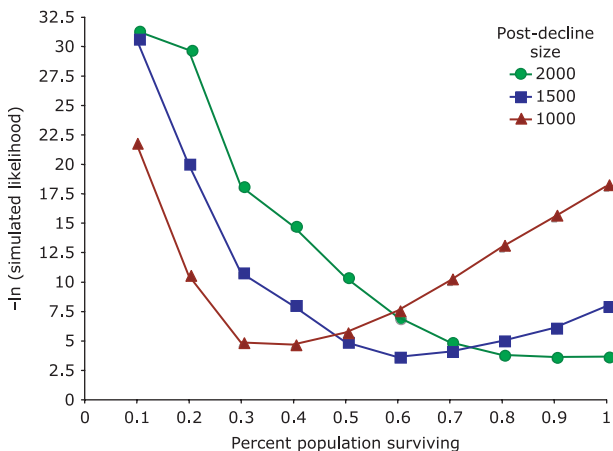


Fig. 3 A plot of $-\ln$ likelihood as a function of percentage surviving population for the penguin ancient genetic data for different initial effective sizes. In all cases, we assume decline starts midway between the ancient and modern sampling.

population decline does increase the likelihood, it does so only marginally for a modern population size of 2000. None of the investigated scenarios (1000, 1500 or 2000) lend support for a significant population size decline at 3000 YBP. We also investigated models that included population expansion between the ancient and modern, which do not fit the observed data at all. Thus, the model with the highest likelihood corresponds to a historical scenario where population size decreases from 2500 to 1500 (a 60% decline) at 3000 YBP.

- 5 *Test the models and perform sensitivity analyses.* The Akaike information criterion (AIC) values (Table 2) reveal that models that include population decline during the last 3000 years fit the data as well as the constant-size model (Δ AIC < 2), despite having more parameters. Thus, these two models are equally likely. In contrast, the model that includes population increase does not fit the data at all and we are able to distinguish between simple and more complex models.

Case Study 2: ancient genetic data from brown bears

Waits *et al.* (1998) demonstrated strong phylogeographical patterns in modern brown bear distribution across Alaska, Canada and the continental USA using mitochondrial

DNA. Since mitochondrial clades were confined to particular geographical regions, Waits *et al.* inferred that the modern distribution of brown bears was a result of multiple colonization events from western Beringia, followed by isolation between these populations. However, Leonard *et al.* (2000) documented the co-occurrence of three geographically distinct modern clades in Alaska over 35 000 YBP, suggesting that the modern phylogenetic signature is relatively recent. Barnes *et al.* (2002) increased ancient genetic sampling spatially and temporally of brown bears. Their results revealed that over the three time periods, they consider (< 35 000, 21 000–35 000 and 21 000–10 000 YBP) brown bears maintain highly divergent mitochondrial DNA clades. Their data thus contradict the possibility of strong modern phylogenetic structure due to recent (post-Last Glacial Maximum) isolation of haplotypes in different geographical regions. Their data also suggest the near absence of bears in the region between 21 000 and 10 000 YBP. Both studies use clade-based inferences. We combine genetic data from three papers (Waits *et al.* 1998; Leonard *et al.* 2000; Barnes *et al.* 2002) to investigate phylogenetic patterns in brown bears. As mentioned earlier, our approach models evolution in the populations and hence is population genetic in flavour. Hence, we do not consider the clades (as demonstrated by the phylogenetic trees) but instead use population genetic summary statistics for our analyses of these data.

Analyses were implemented as described in Box 2 through the following steps:

- 1 *Assign temporal and spatial sampling windows.* The brown bear samples can be subdivided into two geographically separate populations: northwestern US and Canada (or the Pacific Northwest), and Alaska. We divided the data into the same temporal interval that Barnes *et al.* (2002) use ($\geq 35\ 000$, 10 000–21 000, 0–10 000 YBP).
- 2 *Calculate summary statistics.* Using the above temporal and geographical groups, we calculated population genetic summary statistics for the data (Table 3). The number of segregating sites does not change appreciably over time in either population, but the average pairwise difference decreases from ancient to modern in both populations, especially between $\geq 35\ 000$ and 10 000 YBP.
- 3 *Explore simple models.* We began our analyses by simulating the simplest model: a single, constant-sized population

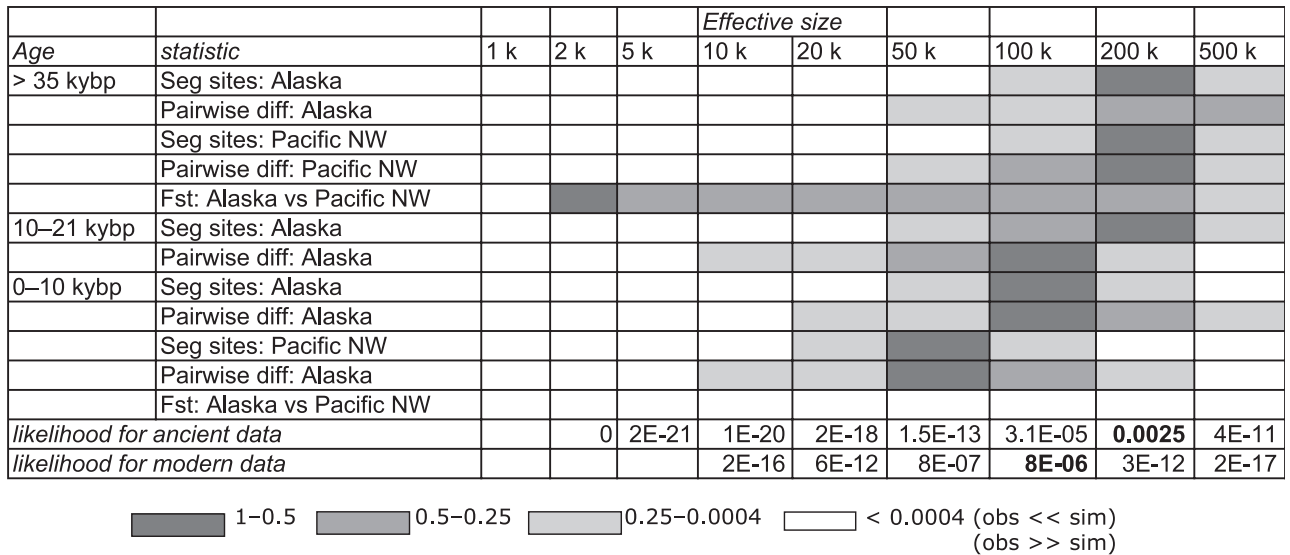


Fig. 4 The probabilities (binned into four categories with darker-coloured cells representing higher probability) for 12 summary statistics (ancient genetic data from brown bears) for different effective sizes, assuming a constant-sized model. None of these models fit the data. We also show the likelihoods for both the ancient and the modern data.

Table 3 Population genetic summary statistics for brown bears across space and time. Both average pairwise difference and segregating sites are shown, with sample size in brackets

	Alaska	Pacific Northwest
> 35 000 YBP	10.21/22 (8)	5.44/12 (8)
10 000–21 000 YBP	2.84/20 (18)	—
Modern–10 000 YBP	4.43/20 (165)	2.19/12 (150)

of brown bears across the Pacific Northwest and Alaska through time. Results from this model help us investigate (i) whether brown bears have a high or low effective size, (ii) whether different time intervals have similar effective sizes, and (iii) whether the interpopulation statistics (F_{ST}) fit single population models. Colour-coded cells (likelihood intervals for each statistic) and the likelihood values for both ancient and modern data in Fig. 4 reveal that (i) in general, brown bears have higher effective sizes (compared to, say the penguin population in the previous data set), (ii) Ancient genetic data have greater likelihoods for higher effective sizes compared to modern genetic data (highest likelihood is for an N_e of 200 000 for ancient data and 100 000 modern data), and (iii) F_{ST} for modern genetic data does not fit a single population model, irrespective of effective size. These inferences suggest population size for brown bears has changed and that two-population models are necessary to explain relatively recent population structure. Notice that we explore single-population models on a logarithmic scale for effective size: 1000, 2000, 5000,

10 000 and so on and we keep increasing the effective size until we are sure that we have found the better-fit parameter space. In summary, this initial exploration suggests that more complex models including two populations should be investigated.

4 Investigate increasingly complex models. Lack of brown bear remains from the Pacific Northwest between 20 000 and 10 000 YBP indicates a possible local extirpation/ or low population size of bears in the region through this time period, an inference also drawn by Leonard *et al.* (2000) and Barnes *et al.* (2002). Synthesizing the single population likelihood explorations and the paleontological observations, we modelled a scenario that included two populations that diverged relatively recently, with the Pacific Northwest population being founded by relatively few individuals following retreat of the ice sheet, and growth to relatively high population size in the modern. This demographic model is represented as an inset in Fig. 5, and includes the following additional parameters: the bottleneck size of the Pacific Northwest brown bears and the divergence time between the Alaskan and the Pacific Northwest (excluding Alaska) brown bears. We base these simulations on an effective population size of 200 000, as this effective size has the highest likelihood for ancient data (including both ancient time intervals). The two-population models fit the observed data much better than the simpler, single-population models. The scenario with the highest likelihood is one in which a very small (≤ 20 founders, 0.001% of the Alaskan population) population diverged 20 000 YBP, and then grew exponentially to recover to a size of 200 000. Fig. 5 also reveals that (i) Pacific Northwest

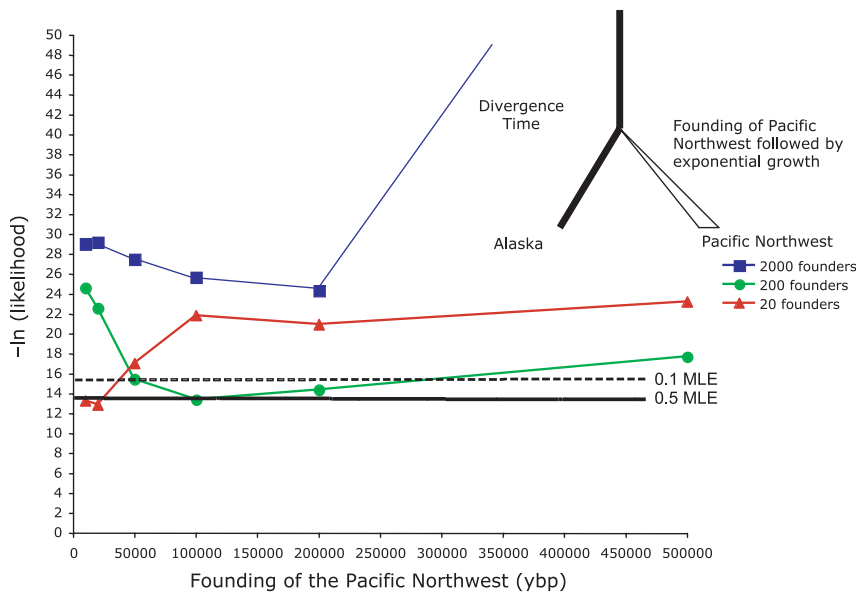


Fig. 5 The $-\ln$ likelihood as a function of divergence time (explored on a logarithmic scale) between the Alaskan and the Pacific Northwest brown bears, including different founding population sizes (for Pacific Northwest bears). The inset illustrates the two-population model. Both the maximum likelihood estimate (MLE) and parameter values with likelihoods within 0.5 and 0.1 of the MLE are shown.

population is founded by very few individuals (likelihoods for higher number of founders in very low), and (ii) do not seem to have much statistical power to differentiate between different divergence times (divergence times between 10 000 and 100 000 ybp have likelihood values within 0.5 of the maximal likelihood value).

- 5 *Test the models and perform sensitivity analyses.* The more complex two-population model involves two additional parameters (bottleneck size and divergence time) compared to the single-population model. However, the single-population models do not fit the data well. We calculate cumulative likelihoods (we assume that the probability of a statistic that is outside the simulated 5000 values is a maximum of 0.0004, since we run the simulation 5000 times and cannot detect likelihoods less than $2/5000$). The most likely effective size for a single-population model is then 100 000, corresponding to a likelihood of 2.6×10^{-10} . The two-population colonization bottleneck model has a much higher likelihood (2.48×10^{-6}), and the AIC values are 46.12 and 31.81 respectively. The AIC value of the two-population models is lower and the Δ AIC (14.3) reveals that it also provides the best statistical fit.

In these two examples, we illustrate the flexibility of using a serial coalescent approach to discriminate between simple and more complex models of population evolution. The flavour of our approach is oriented towards a better understanding of the plausible population historic scenarios, and is not geared towards investigating the exact population parameters that fit the data. Our approach is very user driven and hence, flexible. This flexibility of our approach is its chief strength. Combining our approach with informed knowledge of the species and its local environmental

history can yield statistically rigorous conclusions about population history. Serial SimCoal (and now a Bayesian version, Bayesian Serial SimCoal: www.stanford.edu/group/hadlylab/ssc/BayeSSC.htm) and other applications of the serial coalescent (Drummond & Rambaut 2007) mean that models can accommodate more complex scenarios involving change in size and rates of gene flow between populations, changes in mutation rates, differential sized-populations and multiple events. Although we did not investigate further complexity by using multiple temporal populations, such models are being developed and implemented. These include, for example, metapopulation models (Wakeley & Aliacar 2001).

However, since the approach is so user driven, inappropriate testing of models (e.g. testing more complex models but not simple ones) and inadequate exploration of the parameter space (e.g. not finding the 'optimal' effective size parameter space) could result in misinterpretations of the data. We caution that the absolute likelihood values are not important, as they might change depending on the number of summary statistics used to calculate the likelihood. It is also possible that our approach could yield negative results and not provide any model that fits the data. A more cautious approach might be to apply our method in association with other available methods of analyses and look for concordance between different types of analyses (including our method) for simpler models and parameters (e.g. such as effective population size).

Ecological studies have revealed that populations respond to climatic warming through dispersal to higher latitudes (Root *et al.* 2003), suggesting that gene flow could have been an equally important response to past climatic change (Davis & Shaw 2001). We can now use ancient DNA studies to test probabilities of predictions based on ecological

studies and to directly investigate the importance of dispersal and gene flow in the ability of species to respond to climatic change (Hadly *et al.* 2004). Although such analyses can be complicated, data sets that sample over wide geographical and temporal scales (e.g. Shapiro *et al.* 2004; Debryne *et al.* 2008) are critical to piece together a more dynamic picture of evolutionary history and response to climatic change. Alternative applications of this approach also include testing how particular impacts to populations, such as human hunting (gray whales, Alter 2007) influenced the population size, or exploring when a particular extreme event such as a bottleneck actually occurred (social tuco-tucos, Chan *et al.* 2006). Sensitivity analyses can also be employed to determine which parameters of the models are most, or least, important (Chan *et al.* 2006). Finally, serial coalescent models can also help researchers to determine what type of data (increase in genetic sequence length, increase in genetic samples, more temporal sampling or greater geographical sampling) would increase the likelihood of their results and help narrow the field of events likely to have influenced the history of the population or species.

A potential complication with using a temporal approach is that changes in an earlier time interval will impact the genetics of subsequent intervals. This is because all the samples are part of the same genealogy. Using the modelling approach we describe, it is possible to investigate the influence of past genetic data (and reconstruction of the historic events) on the genetic diversity of the modern populations. Ascertaining the role of historic events and their likelihood on modern population genetic data has not been synthesized at all. Until such synthesis, which requires many more data sets than are presently available, we can only imagine how microevolution has proceeded. We are not yet able to conclude with confidence about the relative dominance of processes in natural populations, how complex demographic history is and how many and frequent are the events structuring genetic diversity of species.

Paleogenomics: going beyond a few hundred base pairs

Apart from providing information on demographic processes in the past, ancient DNA potentially provides the ability to look directly at changes in genes that are potentially under selection, or from genes evolving at different rates. In order to explore the role of selection, we need genetic data from the nuclear genome. While nuclear DNA is difficult to obtain from ancient and historic samples, there are exceptional circumstances under which this type of additional data is preserved. Additionally, modern advances in sequencing techniques like emulsion-based clonal amplification (Margulies *et al.* 2005) assist in producing sequence for many small fragments of nuclear

and mitochondrial DNA. Such methods are ideal for ancient DNA template, which tends to be degraded (Cooper 2006). Recently, such methods have been used to produce billions of sequence base pairs of genetic data for woolly mammoth (Krause *et al.* 2005; Poinar *et al.* 2006; Gilbert *et al.* 2007; Miller *et al.* 2008), Neanderthal (Green *et al.* 2006; Noonan *et al.* 2006; Green *et al.* 2008) and cave bears (Noonan *et al.* 2005). Data on multiple coding nuclear genes will finally allow us to investigate population-level changes in genes that may be under selection. Such research will provide the final link between population genetics and evolution, allowing us to correlate changes in phenotypic traits and the frequencies of the genes that govern them with changes in the environment (e.g. Römler *et al.* 2006). Ancient nuclear DNA has also been used to infer variation in the FOXP2 gene, the transcription factor thought to be involved in the evolution of human language, for Neanderthals (Krause *et al.* 2007), as well as to infer the possible pigmentation status (through the MC1R gene) of Neanderthals (Lalueza-Fox *et al.* 2007, data re-analysed in Coop *et al.* 2008). Introducing the study of selected variation in temporal genetic studies will require analysis of multiple neutral traits, pointing to the relevance of genomic approaches.

Temporal genetic data sets will be made more powerful by the addition of multiple, unlinked neutral markers. Contrasting nuclear and mitochondrial data from the same species over the same time period will lead to a refinement of our ability to discriminate between potential evolutionary histories, and potentially to insights governing the differences between, for example, male vs. female population dynamics within and between species (e.g. Huynen *et al.* 2003).

Although the ability to look at multiple, unlinked nuclear markers will provide an unprecedented ability to track genetic evolution over time, it is important to remember that the technological innovations that allow this (e.g. high throughput sequencing) still have a relatively high error rate. As a result, it is important to concurrently develop statistical methods that deal with potential sequencing errors (Stiller *et al.* 2006; Ho *et al.* 2007) and the effect such errors can have on the inference of demographic history (Axelsson *et al.* 2008; Johnson & Slatkin 2008).

Conclusions

We develop a flexible, simulation-based temporal window approach that allows investigating population historic scenarios that involve more than a single population. We apply our approach to two published ancient DNA data sets to ascertain whether alternative population histories might account for the reported data. Results of our analyses showed that a phylogenetic investigation of these two data sets using serial coalescent simulations enabled us to construct more complex histories to account

for the observed data and to test whether a complex model explained the data more than did a simple one. In one case, we rejected the hypothesis put forward by the authors (penguins) and in other, we concur with the conclusions of the study (brown bears), demonstrating the power of our novel approach.

Our review and re-analysis of published case studies illustrates several points relevant to inferring evolutionary history using phylogenetic data. Analyses revealed that temporal sampling contributes significantly to the power to reconstruct population history (Ramakrishnan *et al.* 2005). Because most ancient DNA studies do not allow extensive temporal and geographical sampling, it is important to use models or analysis frameworks that reflect these biases in sampling. For example, in the penguin data set, additional temporal or geographical sampling might have allowed better reconstruction of history and discrimination between several possible histories. In the brown bear data set, more temporal data and more sequence data would have allowed for a more refined and specific divergence time.

A fundamental aim of the study of evolution is to reveal the genetic and phenotypic response of species and populations to changes in the environment. However, few studies of natural populations allow us to study genetic and phenotypic response over temporal scales most relevant to the evolutionary processes of drift, selection and mutation. Temporal genetic data provide a unique opportunity to discern more complex population histories and potentially to discriminate between alternative population histories to interpret extant genetic diversity patterns within species. Addition of temporal genetic data will enhance our understanding of the effect of past environmental perturbations on population connectivity and population size, and ultimately holds promise for developing conservation predications. Finally, temporal genetic data will allow us to compare ecologically driven (demographic changes, change in gene flow) and evolutionarily driven (changes in mutation rate and selection) population responses of to changes in the environment, and how these responses vary across species. Such research will make significant contributions to an understanding of ecology and its relevance to the evolutionary process.

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