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1 **Combining contemporary and ancient DNA in population genetic and**
 2 **phylogeographic studies**

3
 4 Miguel Navascués^{*†}, Frantz Depaulis[†] and Brent C. Emerson[‡]

5 * INRA, UMR CBGP (INRA/IRD/Cirad/Montpellier SupAgro), Campus
 6 International de Baillarguet, CS 30016, F-34988 Montferrier-sur-Lez Cedex, France.

7 † Laboratoire Ecologie et Evolution, CNRS UMR 7625, UPMC Paris
 8 Universitas, Ecole Normale Supérieure, Paris, France.

9 ‡ Centre for Ecology, Evolution and Conservation, School of Biological Sciences,
 10 University of East Anglia, Norwich NR4 7TJ, United Kingdom.

11
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13
 14 Corresponding author: Brent Emerson, Centre for Ecology, Evolution and
 15 Conservation, School of Biological Sciences, University of East Anglia, Norwich
 16 NR4 7TJ, United Kingdom ph: 0044 (0)1603 592237. fax: 0044(0)1603 592250, e-
 17 mail: b.emerson@uea.ac.uk

18
 19 Running head: Molecular polymorphism analysis of ancient DNA

20
 21 The definitive version is available at www.blackwell-synergy.com

22
 23 Non-standard abbreviations:

24 aDNA: ancient DNA; WF: Wright Fisher model; IMSM: infinitely many sites
 25 (mutational) model; MRCA: Most Recent Common Ancestor; MCMC: Markov chain
 26 Monte Carlo; ABC: approximate Bayesian computation.

27
 28 **Abstract**

29
 30 The analysis of ancient DNA in a population genetic or phylogeographic framework
 31 is an emerging field, as traditional analytical tools were largely developed for the
 32 purpose of analyzing data sampled from a single time point. The analysis of
 33 heterochronous sequence data from closed panmictic populations has received
 34 attention with Markov chain Monte Carlo (MCMC) approaches, but attributing
 35 genetic differences between temporal samples to mutational events between time
 36 points requires the consideration of other factors that may also result in genetic
 37 differentiation. Geographic effects are an obvious factor for species exhibiting
 38 geographic structuring of genetic variation, and departures such as this from a closed
 39 panmictic model require researchers to either exploit software developed for the
 40 analysis of isochronous data, take advantage of simulation approaches using
 41 algorithms developed for heterochronous data, or explore approximate Bayesian
 42 computation. Here we review statistical approaches employed and available software
 43 for the joint analysis of ancient and modern DNA, and where appropriate we suggest
 44 how these may be further developed.

45 **Introduction**

46

47 Non-contemporaneous, or ancient DNA, is providing biologists with new and exciting
48 opportunities to investigate evolutionary pattern and process over a range of temporal
49 scales, from decades (e.g. Harper *et al.* 2006; Martinez-Cruz *et al.* 2007) to hundreds
50 of thousands of years (e.g. Willerslev *et al.* 2007). For the purposes of this paper we
51 define ancient DNA (hereafter aDNA) as DNA recovered from non-ideal biological
52 material – that is to say material that was not preserved or maintained in a manner
53 typically associated with downstream DNA analysis, for which the host organism is
54 no longer alive. Such material includes subfossil remains (typically bones and teeth),
55 archaeological remains, coproliths, mummies, naturally (i.e. not laboratory) frozen
56 remains, ice cores, sediments, museum and herbarium tissues. DNA extracted from
57 such material is typically low quality, with constraints upon both the amount and
58 integrity of the DNA that can be obtained. Nevertheless, there is an increasing volume
59 of studies successfully obtaining samples of aDNA, and frequently these data are
60 being analyzed in a temporal and geographic context (see Ramakrishnan & Hadly
61 2009 for a review). Thus aDNA is becoming increasingly accessible for both
62 population genetic and phylogeographic analysis, offering the possibility of using
63 temporal samples of DNA to characterize population history. However, the analysis
64 of such data is an emerging field, as traditional population genetic and
65 phylogeographic tools were largely developed for the purpose of analyzing data
66 sampled from a single time point, or at the most a sampling interval that spanned no
67 more than a few generations. In this paper we review some of the analytical
68 approaches employed for the joint analysis of ancient and modern DNA, and offer
69 some suggestions for future directions. While we focus on studies utilizing aDNA for
70 temporal sampling, it is important to point out that temporal sampling is not restricted
71 to aDNA. Organisms with short generation times such as viruses offer similar
72 opportunities (e.g. Norja *et al.* 2008; Shackleton *et al.* 2006), but without constraints
73 on DNA quality.

74

75 Perhaps unsurprisingly, the most widely employed marker for phylogeographic and
76 population genetic analyses with temporal sampling over more distant time scales
77 (from hundreds, to thousands of years) is the mitochondrial genome, in particular the
78 fast evolving control region (e.g. Barnett *et al.* 2009; Lambert *et al.* 2002; Shapiro *et al.*
79 *et al.* 2004; Valdiosera *et al.* 2007; Valdiosera *et al.* 2008). Given recent evidence that
80 mtDNA is not a reliable marker for demography (Bazin *et al.* 2006), nuclear data
81 would be a welcome addition, and next generation sequencing technologies offer
82 some promise in this direction. Over more recent time scales of decades,
83 microsatellite markers gain greater prominence, where their high variability provide
84 more potential to reveal demographic events over narrower time scales (e.g. Harper *et al.*
85 *et al.* 2006; Martinez-Cruz *et al.* 2007). However, the utility of microsatellites is not
86 restricted to decadal analyses, and recent efforts have seen the combination of nuclear
87 microsatellites with mtDNA to investigate demographic changes spanning several
88 thousands of years (Keyser-Tracqui *et al.* 2006). Single nucleotide polymorphism
89 (SNP) data offers similar potential and has been used to assess genetic change over a
90 4,000 year period for cattle (Svensson *et al.* 2007).

91

92 As in classical population genetics, the choice of genetic marker for studies
93 incorporating aDNA should be appropriate for the question(s) to be addressed, and in
94 common to all genetic markers are the fundamental population genetic processes that

95 may shape differentiation between heterochronous samples. Population samples of
96 DNA from different time points may vary from subtle changes in allele frequency
97 through to allele loss or gain, due to the processes of mutation, selection, genetic drift
98 and migration. Recent interest has focused on sampling DNA sequences from
99 “measurably evolving” populations (Drummond *et al.* 2003) for which there are
100 sufficiently long or numerous sampled sequences and a fast mutation rate relative to
101 the available range of sequence sampling times. Sequences from such populations
102 have the potential to enable the analysis of temporal changes in the size, structure and
103 substitution rates of populations. Problems have been pointed out about the reliability
104 of aDNA for the estimation of population history, as postmortem DNA damage may
105 act to inflate diversity estimates (Axelsson *et al.* 2008). Distinguishing what is a
106 genuine mutation and what is the consequence of postmortem degradation is thus
107 important for the reliable estimation of population history. A conservative approach is
108 to remove all sites that might represent false, or post mortem induced degradation.
109 Applying this to a data set of steppe bison (*Bison bison/Bison priscus*) previously
110 analyzed by Shapiro *et al.* (2004) results in the removal of signal for population size
111 change observed in the original analyses (Axelsson *et al.* 2008). However, it has been
112 argued that this approach is too conservative, and a subsequent reanalysis
113 implementing a model where sequence variation is the result of a joint process of
114 mutation and postmortem DNA damage is consistent with previous conclusions of
115 population size change over time (Rambaut *et al.* 2009). Clearly there is a need to
116 accommodate mutational artifacts when analyzing aDNA in a population genetic and
117 phylogeographic framework, and recent methods developed to predict errors due to
118 postmortem degradation are a welcome addition (Mateiu & Rannala 2008).

119
120 Phylogeographic analysis of temporally sampled DNA sequences provides for the
121 direct quantification of population turnover within species, with particular reference
122 to climatically mediated regional extinction and recolonization (Benton & Emerson
123 2007; Stewart *et al.* 2009). This is perhaps the most powerful contribution of aDNA to
124 the field of phylogeography, with recent studies suggesting regional population
125 extinction and subsequent recolonization (Barnes *et al.* 2002; Barnett *et al.* 2009;
126 Hofreiter *et al.* 2007; Leonard *et al.* 2007). Temporally sampled DNA sequences also
127 provide the potential for the estimation of substitution rates and divergence times
128 without paleontological calibrations, and there has been much recent interest in the
129 idea that substitution rates within species may be much higher than previously thought
130 (Ho *et al.* 2007; Ho & Larson 2006; Ho *et al.* 2005; Ho *et al.* 2008; Penny 2005).
131 Bayesian Markov chain Monte Carlo (MCMC) analyses of ancient data sets has
132 consistently generated substitution rate estimates exceeding those from the literature.
133 While this acceleration of molecular rates may partially be explained by evolutionary
134 processes (Ho *et al.* 2005) it has been shown that such rate estimates can be biased
135 due to the low information content of aDNA data (Debruyne & Poinar 2009) or
136 demographic model misspecification (Navascués & Emerson 2009). These caveats
137 should be taken into account when estimating molecular rates and in their use to date
138 historical events.

139
140 The concerns raised by Axelsson *et al.* (2008) and the results of Navascués &
141 Emerson (2009) highlight the need for caution when analyzing aDNA in a population
142 genetic or phylogeographic context. Both studies reveal that variation among DNA
143 sequences that originates from processes not accounted for within an underlying
144 evolutionary model may lead to incorrect conclusions regarding evolutionary history.

145 Axelsson *et al.* (2008) demonstrate that when nucleotide differences between
 146 temporal samples arise from postmortem DNA damage they can misleadingly
 147 contribute to demographic inference. Navascués & Emerson (2009) demonstrate that
 148 when nucleotide differences between temporal samples arise not from mutation, but
 149 from other population genetic processes such as genetic drift, immigration or
 150 selection, they can bias divergence and substitution rate estimation. The take home
 151 message is model violation should be given due consideration as a potential
 152 explanation for the result of a model-based analysis. This is not something unique to
 153 population genetic/phylogeographic analyses incorporating aDNA (e.g. Becquet &
 154 Przeworski 2009; Strasburg & Rieseberg 2009).

155
 156 New DNA sequencing technologies promise to deliver greater amounts of aDNA
 157 sequence information for a greater number of taxa (e.g. Allentoft *et al.* 2009; Briggs
 158 *et al.* 2009). While the majority of studies to date have relied on mtDNA sequence
 159 variation, nuclear microsatellites and nuclear coding sequence variation is also within
 160 reach (e.g. Keyser-Tracqui *et al.* 2006; Krause *et al.* 2007; Lalueza-Fox *et al.* 2009;
 161 Lalueza-Fox *et al.* 2008). It is thus both timely and appropriate to evaluate how best
 162 to combine ancient DNA with contemporary DNA in a population genetic and
 163 phylogeographic context. What follows is a review of the statistical tools available,
 164 and suggestions for their future development.

167 **Testing for heterochrony**

168
 169 For the analysis of heterochronous data, one may, as a first step, test if the data show
 170 measurable evolution. If they do not, data may be pooled to proceed with further
 171 analyses using available tools for isochronous data. PATH-O-GENE (Table 1), is a
 172 simple tool that may be used with an input tree, typically a phylogeny reconstructed
 173 without assuming a molecular clock, to compute the correlation between tip to root
 174 distances and sampling times. This provides associated estimates of the mutation rate
 175 and a dated root (the slope and abscissa intercept of the regression) and an output tree
 176 assuming a molecular clock, but taking into account sampling times. It should
 177 however be noted that rigorous testing of such a correlation would require an
 178 additional randomization procedure, since the data do not obey parametric
 179 assumptions.

180
 181 To take into account the potentially confounding effects of factors such as geography
 182 (genetic differentiation resulting from spatial rather than temporal separation) it is
 183 possible to partition genetic variation through the AMOVA (Excoffier *et al.* 1992)
 184 implemented in ARLEQUIN (Table 1). Although originally intended to assess
 185 geographic population structure, AMOVA can also be applied to heterochronous data
 186 partitioned into sampling times, or time bins when pooling is appropriate. This
 187 analysis tests the sampling time effect and estimates the proportion of genetic
 188 variation explained by sampling times. Formally, this is equivalent to testing F_{st}
 189 between sampling times. Indeed, the F_{st} , commonly used to describe differentiation
 190 among populations, is defined as a fixation index and can be seen as a measure of
 191 temporal divergence of populations by drift and not only as a (static) distance between
 192 equilibrium populations. The AMOVA implemented in Arlequin has been applied to
 193 several aDNA data sets (Bramanti *et al.* 2009; Dalen *et al.* 2007; Malmström *et al.*
 194 2009), although these analyses did not allow for the distinction of population effects

195 from temporal ones. In a different approach, Valdiosera *et al.* (2008) applied the
 196 analyses separately on different geological layers to assess geographical structure
 197 through time, though the confounding effect of sampling time may not be entirely
 198 overcome since some layers may be more heterochronous than others (e.g.
 199 Pleistocene *vs* modern). It should be noted that the AMOVA framework was
 200 originally designed for contemporaneous (modern) data and presents several
 201 limitations for the analysis of heterochronous data within the ARLEQUIN
 202 implementation. Ideally factors should be nested, and including both sampling time
 203 and geographic location in an analysis would be more appropriate if a set of sampling
 204 times (or time bins) is found only in a specific location (or conversely if samples from
 205 one population all belong to one chronological layer). An additional constraint is that
 206 the available implementation of AMOVA does not quantitatively take into account
 207 sampling time differences. Excoffier (2007) notes that a population delimitation issue
 208 arises prior to the application of AMOVA and refers to associated attempts through
 209 aggregation techniques. This issue of population delimitation also applies to time
 210 bins. The qualitative feature of the analyses makes it strictly applicable only for
 211 pairwise analyses between two sampling time bins. It would be appropriate for some
 212 studies of modern *vs* ancient data if the latter do not span a substantial time range, but
 213 frequently this is not the case (e.g. Barnes *et al.* 2002; Lambert *et al.* 2002; Shapiro *et*
 214 *al.* 2004). More quantitatively, it is possible to correlate the pairwise genetic distance
 215 with a pairwise time difference through the Mantel test or to both time difference and
 216 geographical distance using a partial Mantel test using IBD (Table 1). Finally, there is
 217 a generalization of AMOVA (GAMOVA) where sampling time can potentially be
 218 treated as a quantitative explanatory cofactor together with other factors (Nievergelt *et*
 219 *al.* 2007), although the procedure has not been applied for this purpose yet.

220
 221 Beyond these statistical tools to test for heterochrony and assess the proportion of
 222 genetic variation explained by sampling time differences, parametric (model based)
 223 statistics offer a powerful complement. Liu and Fu (2007) proposed two classes of
 224 standardized summary statistics aimed at detecting measurable evolution between two
 225 subsets at different times: the first one, D_c , is related to a Nei's (1987) net distance D_a
 226 (see Fig. 1). The other class of statistic, T_c , quantifies the excess of polymorphism
 227 exclusive to one of the two subsets when compared to the isochronous case (i.e. the
 228 null hypothesis being tested). The tests proceed through either (i) parametric
 229 simulation if it is desirable to take into account the stochasticity of the evolutionary
 230 process, or (ii) permutation between the subsets, which may partly lessen confounding
 231 historical effects and would be appropriate to test if samples could be pooled for
 232 subsequent analyses. Both approaches should in general be complementary, the
 233 former being generally more robust. Such statistics are largely inspired from
 234 coalescent theory (Kingman 1982) which is discussed in the next section, in particular
 235 its extension to heterochronous data.

236
 237

238 **Making inferences under the standard neutral model**

239

240 *The serial coalescent*

241

242 Coalescent theory models the genetic history of a sample by probabilistic genealogies
 243 where the nodes represent most recent common ancestors and where the lengths of the
 244 branches - or lineages - are proportional to time (see Wakeley 2008 for a review). The

245 serial (heterochronous) coalescent is a simple extension of the isochronous standard
 246 case (Rodrigo & Felsenstein 1999). Heterochronous data can be described as a list of
 247 subsets, each defined by a sampling time and the associated subset of sequences.
 248 Events occurring on such a genealogy (e.g. coalescence, mutation, recombination,
 249 migration) are limited to lineages that co-occur at the same time point. Thus, at a
 250 given time, the number of extant lineages governs the rate of those events. Analytical
 251 derivations on the serial coalescent may help to predict the heterochrony effect or to
 252 interpret data, to suggest new statistics aimed at investigating heterochrony (see
 253 previous section) and to provide necessary corrections for most available statistics so
 254 as to enable comparison of data sets with different time sampling schemes.

255
 256 Fu (2001) has proposed a diversity-based estimator of mutation rates based on serial
 257 samples; alternatively this method can be used to estimate sample age when an
 258 independent estimate of the mutation rate is available. This approach was
 259 subsequently refined by Liu and Fu (2008). For simplicity Liu and Fu (2008) focused
 260 on a two sampling time case for both the standard neutral model of Wright-Fisher
 261 (WF; Wright 1931), and a model involving any deterministic population size change
 262 starting after the first sampling time. From this approach mean, variance and
 263 covariance is derived for the number of mutations affecting i individuals in a
 264 temporally older sampled subset and j individuals in a more recent sampling.
 265 Although the derivation relates to mutation counts, or segregating sites in the
 266 infinitely many site model (IMSM; Watterson 1975), more general cases with other
 267 mutational models can be estimated with corrected distances. From these counts
 268 commonly used summary statistics are derived (see Fig. 1 for examples) such as the
 269 total number of mutations in the sample, and hence the related Watterson's (1975) θ_W
 270 estimator of polymorphism as well as Tajima's (1983) π diversity estimator.

271 *Testing the standard model*

272
 273
 274 Neutrality test statistics (see Nielsen 2005 for a review) can potentially be corrected
 275 for heterochrony within a general time sampling scheme with an arbitrary number of
 276 sampling times. Assuming WF and IMSM, Forsberg *et al.* (2005) derived the full
 277 probability distribution for the time to the most recent common ancestor of the total
 278 data set, the total length of the genealogy and the length of lineages exclusively
 279 ancestral to ancient samples (see lineages marked with dotted lines in Fig. 1). Such
 280 time quantities (time to MRCA, length of genealogy, length of lineages exclusively
 281 ancestral to ancient samples) can be related to the number of segregating sites, the
 282 number of mutations affecting only ancient samples (note that this may include some
 283 possible fixed differences between ancient and modern samples) and the mutations
 284 shared between ancient and modern samples. Thus, it is possible to perform the
 285 numerical computation of the probabilities to obtain a given summary statistic value
 286 in order to perform a neutrality test.

287
 288 Although the analytical derivation of means and variances of summary statistics is
 289 typically feasible for simple models, deriving their full distribution (as in the work
 290 described in the previous paragraph) is generally more challenging. Simulation
 291 approaches provide a sensible alternative to test evolutionary models or for parameter
 292 inference. Coalescent simulations are commonly used to empirically investigate the
 293 distribution of polymorphisms under flexible evolutionary scenarios, to test those
 294 scenarios through comparison with data, or make inferences for associated parameters

295 in a more refined approach such as MCMC and ABC (see latter section). Simulations
 296 also allow for flexibility of model specification and provide efficient simulation
 297 schemes because one need only consider explicitly the history of a sample, and only
 298 at times where events modify that history, such as common ancestry, mutation,
 299 recombination or migration. Simulation within the serial coalescent algorithm is a
 300 straightforward minor modification of the contemporaneous case (Achaz *et al.* 2004;
 301 Anderson *et al.* 2005; Depaulis *et al.* 2009) and provides a powerful tool to assess
 302 heterochrony effects, test evolutionary scenarios, design appropriate sampling
 303 schemes, and investigate the statistical properties of available methods.

304
 305 In the context of assessing the effects of heterochrony on neutrality test statistics,
 306 coalescent simulation has recently been used to reveal that heterochrony can introduce
 307 substantial bias to parameter estimation from summary statistics (Depaulis *et al.*
 308 2009). Heterochrony increases coalescence times by increasing the difference
 309 between sampling times, since the ancestral lineage of the youngest individual must
 310 first reach the sampling point of the ancient one before the two lineages can coalesce.
 311 This leads to lengthening of a genealogy, and the overestimation of polymorphism
 312 with classical estimators such as diversity π (Fig. 1), indicating that direct comparison
 313 of polymorphism between data sets with sampled from different time points is
 314 inappropriate. Using a large aDNA data set for cave bears, heterochrony has been
 315 shown to strongly influence the conclusions of several neutrality tests (Depaulis *et al.*
 316 2009). A straightforward correction factor can however be easily implemented for the
 317 diversity estimator and between population distances (Depaulis *et al.* 2009). For
 318 moderate heterochrony (sampling time differences below N_e generations), a likely
 319 scenario for most available aDNA data, terminal branches (i.e. directly leading to
 320 sampled tips) tend to be proportionally more affected. This leads to star like trees, an
 321 excess of mutations with low frequency in the sample, as revealed by negative
 322 Tajima's D , and a deficit of associations between mutations (linkage disequilibrium
 323 statistics, being sensitive to mutation frequency and the shape of the tree, provide
 324 information even in the absence of recombination). The result of this is the spurious
 325 mimicry of typical signatures of demographic processes such as population
 326 expansion. Both Achaz *et al.* (2004) and Depaulis *et al.* (2009) have shown through
 327 simulation that heterochrony can lead to substantial genetic differentiation between
 328 sampling points or, more generally, between data sets with different time sampling
 329 schemes. A useful consequence of this is that such differentiation may be used to
 330 estimate the rate of drift and thus, the effective population size (Achaz *et al.* 2004).

331
 332

333 **Making inferences under complex models**

334

335 *Likelihood and Bayesian inference using MCMC genealogy sampling*

336

337 The likelihood of a given parameter value is a function proportional to the probability
 338 of the data given the parameter value, $L(\theta|D)=\alpha P(D|\theta)$ (we will ignore the constant of
 339 proportionality α from this point). This function is used in statistical modelling to
 340 make inferences about the parameter values of a model. One approach to make
 341 inferences is to quantify the parameter value that maximizes the likelihood function
 342 (maximum likelihood estimate) and use the likelihood function profile around that
 343 value to determine the confidence intervals for the estimate. A Bayesian alternative
 344 approach is to combine the likelihood with some prior probability (information or

345 belief prior to the experiment) to obtain the posterior probability distribution, and use
 346 this distribution to obtain a point estimate and credible intervals of the model
 347 parameter (see Beaumont & Rannala 2004 for further details).

348

349 It is possible to calculate the probability of a genealogy given a demographic model,
 350 $P(G|\theta)$, by using the coalescent as a model (Felsenstein 1992). Additionally,
 351 calculating the probability of the data (genetic state of a sample) given a genealogy,
 352 $P(D|G)$ has been established from the field of phylogenetics (Felsenstein 1981). The
 353 genealogy of the sample is unknown; thus, in order to derive the likelihood, it is
 354 necessary to integrate over all possible genealogies, $L(\theta|D) = \sum_G P(D|G)P(G|\theta)$.

355 This will be the likelihood of full data. In practice, only a selection of random
 356 genealogies will be used in this integration, because the number of possible
 357 genealogies is infinite (a large number of topologies and an infinite combination of
 358 branch lengths). However, most of the possible genealogies will be very unlikely and
 359 will contribute little to the estimation of the likelihood. Therefore, it is desirable to
 360 sample the genealogies in an efficient way, i.e. favouring plausible genealogies.
 361 Currently, the most popular framework to sample genealogies efficiently is the
 362 Markov chain Monte Carlo (MCMC) approach, in an implementation originally
 363 proposed by Kuhner *et al.* (1995). This method consists of a random walk over the
 364 space of the model parameter values and over possible genealogies, and it is designed
 365 in a way that it should visit each point of that space proportionally to its likelihood
 366 (see Wakeley 2008 for further details and a review of various implementations). This
 367 approach has been implemented in several software packages targeting different
 368 demographic scenarios (see Kuhner 2009 for a review); among them only BEAST
 369 (Table 1) allows for the sampling of genealogies of genes collected at different time
 370 points (Drummond *et al.* 2002) or a model of DNA damage for aDNA (Rambaut *et al.*
 371 2009).

372

373 The initial interest in developing BEAST was to take advantage of heterochronous
 374 data to estimate mutation rates (Drummond *et al.* 2002). In the case of isochronous
 375 data, substitution rates are frequently estimated from a phylogeny using fossil or
 376 geological data to calibrate node dates. For heterochronous data, it is possible to
 377 estimate the number of mutations occurring in the time interval between samples
 378 (and, thus, the mutation rate) without using any external data to calibrate the
 379 genealogy (see Drummond *et al.* 2003 for further details). This potentially allows one
 380 to obtain species-specific or population-specific molecular rate estimates that cannot
 381 usually be obtained from phylogenies because of the difficulty of assigning fossils to
 382 a particular lineage below the genus level. The MCMC method to estimate the
 383 molecular rate implemented in BEAST was first used in the analysis of Adélie
 384 penguin (*Pygoscelis adeliae*) modern and ancient DNA samples (Lambert *et al.* 2002)
 385 and has subsequently been applied to a number of other aDNA data sets (e.g. Ho *et al.*
 386 2007), usually providing rate estimates higher than those obtained from phylogenies
 387 (this has led to the debate mentioned in the introduction).

388

389 Analysis of complex historical change in population size can be addressed in BEAST
 390 using the Bayesian skyline plot framework (Drummond *et al.* 2005). This method is
 391 based on the classical skyline plot analysis (Pybus *et al.* 2000), in which population
 392 size is estimated from consecutive time intervals separated by coalescent events from
 393 a given genealogy (in practice, a maximum likelihood estimate of the genealogy). An

394 estimate of population size is obtained for each time interval from its length, based on
395 the expected coalescence time for the number of lineages present at in that time
396 interval. The graphical representation of this piecewise demographic model yields a
397 plot that evokes the skyline of a city (Fig. 2b). The Bayesian skyline plot is an
398 extension of this approach with the following modification: (i) time intervals of the
399 piecewise demographic model do not need to be defined by consecutive coalescent
400 events (generalised skyline plot by Strimmer & Pybus 2001); (ii) a prior is used to
401 make population sizes of consecutive time intervals correlated; and (iii) the
402 uncertainty of the genealogy is taken into account with the MCMC algorithm for
403 sampling genealogies (Drummond *et al.* 2005). Although the model parameters are
404 the population sizes at each interval, and the times defining the intervals, posterior
405 probabilities for them are ignored in the output of BEAST. Instead, a plot of the
406 estimated population size and the associated credibility interval as a function of time
407 is presented (Fig. 2c), which is more informative about the past demographic history
408 than the model parameter values. The analysis of heterochronous steppe bison
409 mtDNA data remains an enlightening example of this analysis and its development
410 (Drummond *et al.* 2005; Rambaut *et al.* 2009; Shapiro *et al.* 2004).

411
412 The coalescent model implemented within BEAST was (until recently) that of a single
413 population, and, thus, did not allow one to address the study of spatially structured
414 populations. Potential problems of imposing spatially structured heterochronous data
415 onto such a model for the estimation of mutation rate have been pointed out
416 (Navascués & Emerson 2009), and although it has not been evaluated, similar issues
417 may exist for demographic inference. However, two recent developments have
418 opened the door to phylogeographical analyses with BEAST. First, Lemey *et al.*
419 (2009) have introduced a Bayesian modelling of character evolution for the inference
420 of ancestral states. Using geographical locations as character states, Lemey *et al.*
421 (2009) advocate the inference of posterior probabilities for the location (state) of
422 ancestral nodes and migration events (changes of state). This is a significant
423 improvement over classical phylogeographical studies (e.g. Hofreiter *et al.* 2004;
424 Leonard *et al.* 2000) for two reasons: (i) the uncertainty on the genealogy
425 reconstruction is taken into account (classical phylogeography is frequently based on
426 a single ‘phylogeny’) and (ii) there is a rigorous statistical interpretation of the results
427 (classical phylogeography is frequently reduced to a visual description of the
428 geographical distribution of lineages). Nevertheless, it must be noted that the use of
429 this model of character evolution does not change the model of the coalescent, which
430 remains constrained to that of a single panmictic population and it is not clear whether
431 this would be robust to some population structure scenarios. The second development
432 is the implementation of the multispecies coalescent within BEAST by Heled &
433 Drummond (2009). In this new method (named *BEAST) a set of species is
434 considered to diverge (by successive bifurcations, following a birth-death model)
435 from a common ancestral species. Divergence times and effective population sizes for
436 each contemporary and ancestral species (i.e. the ‘species tree’) determine the
437 coalescent probabilities used in the sampling of genealogies. The MCMC scheme
438 works by integrating over gene genealogies and over species tree topologies to obtain
439 estimates of the posterior probability distribution of the species tree (gene genealogies
440 have only the role of a ‘nuisance parameter’ and are ignored in the output). The
441 purpose of the method is to reconstruct the phylogeny from multiple loci. In
442 particular, it addresses the problem of incongruent gene genealogy topologies among
443 loci and with the species tree topology due to incomplete lineage sorting by using a

444 coalescent model. Although the method is focused on phylogeny reconstruction, it can
 445 be applied to a set of populations to determine the 'population tree' and use it to make
 446 phylogeographical inferences, i.e. inferring and dating the vicariance or colonization
 447 events responsible of the divergence of those populations.

448
 449 Estimating the likelihood distribution from full data with the MCMC algorithm is in
 450 principle the best way to extract most of the information contained within the data.
 451 BEAST is currently the only implementation of this method for heterochronous data
 452 but unfortunately it only includes single population demographic models, divergence
 453 models without migration (*BEAST) and coalescent models without intragenic
 454 recombination. Regarding the lack of intragenic recombination in the coalescent
 455 model, this has not been considered a problem in the past, as most aDNA studies have
 456 targeted mitochondrial DNA (see Ramakrishnan & Hadly 2009 for a review).
 457 However, new sequencing technologies are a promising tool for the characterization
 458 of nuclear genetic diversity in aDNA (Millar *et al.* 2008) and future studies would
 459 likely benefit from the implementation of recombination. These features may be
 460 available in future versions or new programs of MCMC coalescent sampling, but
 461 efficiently incorporating recombination into MCMC presents many challenges – in
 462 particular likelihood surfaces become very rugged and difficult to explore. As an
 463 alternative researchers may use alternative methods for the estimation of the
 464 likelihood based on summary statistics rather than on full data.

465 *Approximating the likelihood using summary statistics*

466
 467 Estimating the likelihood of full data, $L(\theta|D)$, is computationally intensive, the
 468 methods are difficult to implement in a computer program, and these difficulties
 469 increase with model complexity and the size of a given data set. Alternatively, it
 470 is possible to estimate the likelihood of a subset of the information within the data
 471 contained in summary statistics, S : $L(\theta|S)$. This approach takes advantage of the ease
 472 of simulating pseudo-samples using the coalescent model and computing summary
 473 statistics for those pseudo-samples. The general idea consist in simulating pseudo-
 474 samples under a range of parameter values and rejecting those simulations yielding
 475 summary statistics very different (using a predetermined threshold) to the summary
 476 statistics of the target sample. The proportion of accepted simulations is used as an
 477 approximation of the likelihood. This approximation of likelihoods is most frequently
 478 used in the Bayesian framework in what has been termed Approximate Bayesian
 479 Computation (ABC). Specifically, the model parameter values are taken from some
 480 prior distributions for performing the simulations. Simulations are rejected or
 481 accepted in the same way as described above. The distribution of parameter values
 482 from the accepted simulations is used as an approximation of the posterior distribution
 483 (e.g. see Pritchard *et al.* 1999). The estimates obtained from this method are highly
 484 dependent on the arbitrarily chosen threshold value for the rejection step: values that
 485 are too large will increase the error by accepting pseudo-samples far from real
 486 sample; values that are too small will require a prohibitively large number of
 487 simulations in order to accept enough simulations for the approximation of the
 488 posterior distribution. In order to address this problem, Beaumont *et al.* (2002)
 489 proposed an additional regression step after the rejection. In this step a linear
 490 regression of the parameter values as a function of the summary statistics is calculated
 491 from the accepted simulations. The parameter values are then adjusted using the
 492 regression and the posterior distribution is estimated from the adjusted values. This
 493

494 adjustment is termed the regression algorithm and has been responsible for the recent
 495 success of ABC in population genetics because it allows accurate inferences with an
 496 affordable number of simulations (Fig. 3).

497

498 ABC methods have been little used in aDNA phylogeographic and population
 499 genetics. One of the reasons for this could be the lack of user-friendly software to
 500 perform all the necessary steps of the analysis (but see DIY ABC; Table 1). However,
 501 there are at least three coalescent simulators (SERIAL SIMCOAL, COMPASS and
 502 NETRECODON; Table 1) that perform coalescent simulations of heterochronous data.
 503 With a minimal knowledge of programming, they can be used for the simulation step
 504 of the ABC and their output can be analysed in R (with the functions available from
 505 Mark Beaumont or SERIAL SIMCOAL websites, see Table 1) for the rejection and
 506 regression steps. This was the procedure followed by Chan *et al.* (2006) to
 507 characterize a bottleneck for the rodent *Ctenomys sociabilis* from aDNA and by
 508 Ghirotto *et al.* (in press) to study the genetic continuity of bronze-age and modern
 509 human populations of Sardinia; both works using SERIAL SIMCOAL and R to perform
 510 the analyses. A simple example for using COMPASS and R for ABC analysis can be
 511 found as supplementary material (it will reproduce the analyses presented in Fig. 3).

512

513 With the demographic and mutational model flexibility of the currently available
 514 coalescent simulators, there is much potential in an ABC approach for the analysis of
 515 heterochronous DNA sequence data. This is particularly relevant for addressing
 516 models outside the scope of BEAST, such as structured populations with migration or
 517 genes with intragenic recombination, for which no other alternative is currently
 518 available. However, a note of caution is necessary. With so much flexibility one might
 519 be tempted to fit a complex model to data with low information content. As in any
 520 model-based inference, special care should be taken when choosing the proposed
 521 model and the posterior and prior distributions should be compared. It is also
 522 advisable to evaluate only a few different models and use a procedure of model
 523 selection (such as that proposed with an R function by Beaumont (2006)) to estimate
 524 the posterior probability for each model.

525

526 *Model testing and model selection*

527

528 Estimating the parameters of a single demographic model will not suffice in most
 529 study cases for which several demographic models are plausible and should be
 530 considered. Thus, it is necessary to be able to test the models or measure the goodness
 531 of fit of the models to the data. Coalescent simulation may be used to test particular
 532 evolutionary scenarios within a range of parameter values of interest for
 533 heterochronous data. Several aDNA studies have made use of SERIAL SIMCOAL to
 534 test different demographic hypothesis: genealogic continuity between ancient and
 535 modern populations (e.g. Belle *et al.* 2006 and Bramanti *et al.* 2009), demographic
 536 changes (e.g. Ramakrishan *et al.* 2005; Valdiosera *et al.* 2008) or population structure
 537 (Fabre *et al.* 2009 (doi: 10.1371/journal.pone.0005151)). The general procedure
 538 consist in choosing a set of models, fixing a single value for each parameter of the
 539 model (or few combinations of values) and simulating a large number of pseudo-
 540 samples for each model. The testing of each model (for a fixed combination of
 541 parameter values) is performed by estimating p-values from the distributions of the
 542 summary statistic (see Guimaraes *et al.* 2009 (doi: 10.1093/molbev/msp126) for a test
 543 statistic combining the p-values estimated from different summary statistics). An

544 important drawback of this approach is that it requires a large number of simulations
545 to estimate the p-value for each combination of parameter values considered, which
546 severely limits the range of parameter values that can be explored. In order to select
547 the model with the best fit to the data, Belle et al (2009, doi: 10.1038/hdy.2008.103)
548 propose to rank the models by the number of summary statistic for which the
549 estimated p-value is lower than the threshold for significance. Although this ranking
550 gives an idea of the plausibility of the different models it is a poor measure of
551 goodness of fit because some summary statistics are expected to be correlated and the
552 'measure' is not quantitative to the departure from the model. Alternatively,
553 Ramakrishnan and Hadley (2009) propose to calculate the Akaike information
554 criterion using a crude approximation of the likelihood based on the estimated p-
555 values. Despite their past popularity, these methods suffer from serious limitations. It
556 is our view that this kind of approach will be superseded by the ABC methods
557 described in the previous section, as they allow to explore each model for a
558 continuous range of parameter values (instead of a single or few fixed values) and
559 offer more statistically rigorous procedures for model selection (see Ghirotto et al. in
560 press) as an example for model selection procedures in ABC).

561

562

563 **Concluding remarks**

564

565 Ancient DNA is being incorporated in population genetic and phylogeographic
566 analyses with increasing frequency. As such, careful consideration is required by
567 researchers regarding available statistical approaches, and the appropriateness of these
568 for the data to be analysed. Approaches are available to assess the influence of
569 evolutionary change on sequences across sampling intervals, and to control for other
570 variables, such as geographic location, that may contribute to genetic differentiation
571 among samples of sequences. While these approaches currently present some
572 limitations, it would seem that these could probably be overcome with further
573 development of existing tools. The analyses available to researchers are perhaps less
574 limited than they might first appear, but to increase flexibility researchers are required
575 to explore options beyond stand alone analytical packages. For the analysis of single
576 populations conforming to panmixia, a full-data likelihood approach as implemented
577 in BEAST offers a versatile tool to make demographic inferences, and recent
578 developments open the door for phylogeographic analysis with multiple population
579 demographic inference (Heled & Drummond 2009; Lemey *et al.* 2009). For aDNA
580 studies where sampled populations deviate from models available within BEAST (e.g.
581 structured populations, gene flow, recombination), summary statistics and their
582 approximation in a Bayesian framework provide for an alternative approach. However
583 for all model-based analyses researchers are advised to take a cautious approach to the
584 interpretation of their results by comparing posterior and prior parameter values,
585 considering model violation, and the potential consequences of lack of information
586 within heterochronously sampled data. We echo previous calls for the need for rigor
587 in aDNA analysis (Cooper & Poinar 2000), but extend this call to the downstream
588 analysis of this data for historical inference.

589 **References**

- 590 Achaz G, Palmer S, Kearney M, *et al.* (2004) A robust measure of HIV-1
591 population turnover within chronically infected individuals. *Mol Biol*
592 *Evol*, **21**, 1902-1912.
- 593 Allentoft ME, Schuster SC, Holdaway RN, *et al.* (2009) Identification of
594 microsatellites from an extinct moa species using high-throughput
595 (454) sequence data. *Biotechniques*, **46**, 195-200.
- 596 Anderson CN, Ramakrishnan U, Chan YL, Hadly EA (2005) Serial SimCoal: a
597 population genetics model for data from multiple populations and
598 points in time. *Bioinformatics*, **21**, 1733-1734.
- 599 Axelsson E, Willerslev E, Gilbert MTP, Nielsen R (2008) The effect of ancient
600 DNA damage on inferences of demographic histories. *Molecular Biology*
601 *and Evolution*, **25**, 2181-2187.
- 602 Barnes I, Matheus P, Shapiro B, Jensen D, Cooper A (2002) Dynamics of
603 Pleistocene population extinctions in Beringian brown bears. *Science*,
604 **295**, 2267-2270.
- 605 Barnett R, Shapiro B, Barnes I, *et al.* (2009) Phylogeography of lions (*Panthera*
606 *leo* ssp.) reveals three distinct taxa and a late Pleistocene reduction in
607 genetic diversity. *Molecular Ecology*, **18**, 1668-1677.
- 608 Barton NH, Depaulis F, Etheridge AM (2002) Neutral evolution in spatially
609 continuous populations. *Theoretical Population Biology*, **61**, 31-48.
- 610 Bazin E, Glemin S, Galtier N (2006) Population size does not influence
611 mitochondrial genetic diversity in animals. *Science*, **312**, 570-572.
- 612 Beaumont M, Rannala B (2004) The Bayesian revolution in genetics. *Nature*
613 *Reviews Genetics*, **5**, 251-261.
- 614 Beaumont MA, Matsumura S, Forster P, Renfrew C (2006) Joint determination
615 of topology, divergence time, and immigration in population trees. In:
616 *Simulation, Genetics, and Human Prehistory*. McDonald Institute for
617 Archaeological Research, Cambridge.
- 618 Beaumont MA, Zhang W, Balding DJ (2002) Approximate Bayesian
619 computation in population genetics. *Genetics*, **162**, 2025-2035-2025-
620 2035.
- 621 Becquet C, Przeworski M (2009) Learning about modes of speciation by
622 computational approaches. *Evolution*, **63**, 2547-2562.
- 623 Benton MJ, Emerson BC (2007) How did life become so diverse? The
624 dynamics of diversification according to the fossil record and
625 molecular phylogenetics. *Palaeontology*, **50**, 1-19.
- 626 Bramanti B, Thomas MG, Haak W, *et al.* (2009) Genetic discontinuity between
627 local hunter-gatherers and central Europe's first farmers. *Science*, **326**,
628 137-140.
- 629 Briggs AW, Good JM, Green RE, *et al.* (2009) Targeted Retrieval and Analysis
630 of Five Neandertal mtDNA Genomes. *Science*, **325**, 318-321.

- 631 Chan YL, Anderson CNK, Hadly EA (2006) Bayesian estimation of the timing
632 and severity of a population bottleneck from ancient DNA. *PLoS*
633 *Genetics*, **2**, 451-460.
- 634 Cooper A, Poinar HN (2000) Ancient DNA: do it right or not at all. *Science*,
635 **289**, 1139-1139.
- 636 Dalen L, Nystrom V, Valdiosera C, *et al.* (2007) Ancient DNA reveals lack of
637 postglacial habitat tracking in the arctic fox. *Proceedings of the National*
638 *Academy of Science, USA*, **104**, 6726-6729.
- 639 Debruyne R, Poinar HN (2009) Time dependency of molecular rates in ancient
640 DNA data sets, a sampling artifact? *Systematic Biology*, **58**, 348-359.
- 641 Depaulis F, Orlando L, Hänni C (2009) Using classical population genetic
642 tools with heterochronous data: time matters! *PLoS ONE*, **in press**.
- 643 Drummond AJ, Nicholls GK, Rodrigo AG, Solomon W (2002) Estimating
644 mutation parameters, population history and genealogy
645 simultaneously from temporally spaced sequence data. *Genetics*, **161**,
646 1307-1320.
- 647 Drummond AJ, Pybus OG, Rambaut A, Forsberg R, Rodrigo AG (2003)
648 Measurably evolving populations. *Trends in Ecology and Evolution*, **18**,
649 481-488.
- 650 Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent
651 inference of past population dynamics from molecular sequences.
652 *Molecular Biology and Evolution*, **22**, 1185-1192.
- 653 Epperson BK (1999) Gene Genealogies in Geographically Structured
654 Populations. *Genetics*, **152**, 797-806.
- 655 Excoffier L (2007) Analysis of population subdivision. In: *Handbook of*
656 *statistical genetics* (eds. Balding DJ, Bishop M, Cannings C). Wiley,
657 Chichester.
- 658 Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance
659 inferred from metric distances among DNA haplotypes: application to
660 human mitochondrial DNA restriction data. *Genetics*, **131**, 479-491.
- 661 Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum
662 likelihood approach. *Journal of Molecular Evolution*, **17**, 368-376-368-376.
- 663 Felsenstein J (1992) Estimating effective population size from samples of
664 sequences: inefficiency of pairwise and segregating sites as compared
665 to phylogenetic estimates. *Genetics Research*, **59**, 139-147-139-147.
- 666 Forsberg R, Drummond AJ, Hein J (2005) Tree measures and the number of
667 segregating sites in time-structured population samples. *BMC Genetics*,
668 **6**, doi:10.1186/1471-2156-1186-1135.
- 669 Fu YX (2001) Estimating mutation rate and generation time from longitudinal
670 samples of DNA sequences. *Molecular Biology and Evolution*, **18**, 620-626.
- 671 Harper GL, Maclean N, Goulson D (2006) Analysis of museum specimens
672 suggests extreme genetic drift in the adonis blue butterfly

- 673 (Polyommatus bellargus). *Biological Journal of the Linnean Society*, **88**,
674 447-452.
- 675 Heled J, Drummond AJ (2009) Bayesian inference of species trees from
676 multilocus data. *Molecular Biology and Evolution*, **in press**.
- 677 Ho SYW, Kolokotronis S-O, Allaby RG (2007) Elevated substitution rates
678 estimated from ancient DNA sequences. *Biology Letters*, **3**.
- 679 Ho SYW, Larson G (2006) Molecular clocks: when times are a-changin'. *Trends*
680 *in Genetics*, **22**, 79-83.
- 681 Ho SYW, Phillips MJ, Cooper A, Drummond AJ (2005) Time dependency of
682 molecular rate estimates and systematic overestimation of recent
683 divergence times. *Molecular Biology and Evolution*, **22**, 1561-1568.
- 684 Ho SYW, Saarma U, Barnett R, Haile J, Shapiro B (2008) The effect of
685 inappropriate calibration: Three case studies in molecular ecology.
686 *PLoS ONE*, **3**, e1615.
- 687 Hofreiter M, Muenzel S, Conard NJ, *et al.* (2007) Sudden replacement of cave
688 bear mitochondrial DNA in the late Pleistocene. *Current Biology*, **17**,
689 R122-R123.
- 690 Hofreiter M, Rabeder G, Jaenicke-Despres V, *et al.* (2004) Evidence for
691 reproductive isolation between cave bear populations. *Curr Biol*, **14**, 40-
692 43.
- 693 Keyser-Tracqui C, Crubezy E, Pamzav H, Varga T, Ludes B (2006)
694 Population origins in Mongolia: Genetic structure analysis of ancient
695 and modern DNA. *American Journal of Physical Anthropology*, **131**, 272-
696 281.
- 697 Kingman JFC (1982) The coalescent. *Stochastic Processes and their Applications*,
698 **13**, 235-248.
- 699 Krause J, Lalueza-Fox C, Orlando L, *et al.* (2007) The derived FOXP2 variant of
700 modern humans was shared with neandertals. *Current Biology*, **17**,
701 1908-1912.
- 702 Kuhner MK (2009) Coalescent genealogy samplers: windows into population
703 history. *Trends in Ecology & Evolution*, **24**, 86-93-86-93.
- 704 Kuhner MK, Yamato J, Felsenstein J (1995) Estimating effective population
705 size and mutation rate from sequence data using Metropolis-Hastings
706 sampling. *Genetics*, **140**, 1421-1430-1421-1430.
- 707 Lalueza-Fox C, Gigli E, de la Rasilla M, Fortea J, Rosas A (2009) Bitter taste
708 perception in Neanderthals through the analysis of the *TAS2R38* gene.
709 *Biology Letters*, **doi: 10.1098/rsbl.2009.0532**.
- 710 Lalueza-Fox C, Gigli E, de la Rasilla M, *et al.* (2008) Genetic characterization of
711 the ABO blood group in Neandertals. *BMC Evolutionary Biology*, **8**.
- 712 Lambert DM, Ritchie PA, Millar CD, *et al.* (2002) Rates of evolution in ancient
713 DNA from Adelie penguins. *Science*, **295**, 2270-2273.

- 714 Lemey P, Rambaut A, Drummond A, Suchard MA (2009) Bayesian
 715 phylogeography finds its roots. *PLoS Computational Biology*, **5**,
 716 e1000520.
- 717 Leonard JA, Vila C, Fox-Dobbs K, *et al.* (2007) Megafaunal extinctions and the
 718 disappearance of a specialized wolf ecomorph. *Current Biology*, **17**,
 719 1146-1150.
- 720 Leonard JA, Wayne RK, Cooper A (2000) Population genetics of Ice Age
 721 brown bears. *Proceedings of the National Academy of Sciences of the United*
 722 *States of America*, **97**, 1651-1654.
- 723 Liu X, Fu YX (2007) Test of genetical isochronism for longitudinal samples of
 724 DNA sequences. *Genetics*, **176**, 327-342.
- 725 Liu X, Fu YX (2008) Summary statistics of neutral mutations in longitudinal
 726 DNA samples. *Theor Popul Biol*, **74**, 56-67.
- 727 Malmström H, Gilbert MT, Thomas MG, *et al.* (2009) Ancient DNA reveals
 728 lack of continuity between neolithic hunter-gatherers and
 729 contemporary Scandinavians. *Curr Biol*, **19**, 1758-1762.
- 730 Martinez-Cruz B, Godoy JA, Negro JJ (2007) Population fragmentation leads
 731 to spatial and temporal genetic structure in the endangered Spanish
 732 imperial eagle. *Molecular Ecology*, **16**, 477-486.
- 733 Mateiu LM, Rannala BH (2008) Bayesian inference of errors in ancient DNA
 734 caused by postmortem degradation. *Molecular Biology and Evolution*, **25**,
 735 1503-1511.
- 736 Millar CD, Huynen L, Subramanian S, Mohandesan E, Lambert DM (2008)
 737 New developments in ancient genomics. *Trends in Ecology & Evolution*,
 738 **23**, 386-393.
- 739 Navascués M, Emerson BC (2009) Elevated substitution rate estimates from
 740 ancient DNA: model violation and bias of Bayesian methods. *Molecular*
 741 *Ecology*, **18**, 4390-4397.
- 742 Nei M (1987) *Molecular evolutionary genetics* Columbia University Press, New
 743 York.
- 744 Nievergelt CM, Libiger O, Schork NJ (2007) Generalized analysis of molecular
 745 variance. *PLoS Genetics*, **3**, e51.
- 746 Norja P, Eis-Hubinger AM, Soderlund-Venermo M, Hedman K, Simmonds P
 747 (2008) Rapid sequence change and geographical spread of human
 748 parvovirus B19: Comparison of B19 virus evolution in acute and
 749 persistent infections. *Journal of Virology*, **82**, 6427-6433.
- 750 Penny D (2005) Evolutionary biology - relativity for molecular clocks. *Nature*,
 751 **436**, 183-184.
- 752 Pritchard JK, Seielstad MT, Perez-Lezaun A, Feldman MW (1999) Population
 753 growth of human Y chromosomes: a study of Y chromosome
 754 microsatellites. *Molecular Biology and Evolution*, **16**, 1791-1798-1791-
 755 1798.

- 756 Pybus OG, Rambaut A, Harvey PH (2000) An integrated framework for the
757 inference of viral population history from reconstructed genealogies.
758 *Genetics*, **155**, 1429–1437–1429–1437.
- 759 Ramakrishnan U, Hadly EA (2009) Using phylochronology to reveal cryptic
760 population histories: review and synthesis of 29 ancient DNA studies.
761 *Molecular Ecology*, **18**, 1310–1330–1310–1330.
- 762 Ramakrishnan U, Hadly EA, Mountain JL (2005) Detecting past population
763 bottlenecks using temporal genetic data. *Molecular Ecology*, **14**, 2915-
764 2922.
- 765 Rambaut A, Ho SYW, Drummond A, Shapiro B (2009) Accommodating the
766 effect of ancient DNA damage on inferences of demographic histories.
767 *Molecular Biology and Evolution*, **26**, 245-248.
- 768 Rodrigo AG, Felsenstein J (1999) Coalescent Approaches to HIV Population
769 Genetics. In: *The Evolution of HIV* (ed. Crandall K). Johns Hopkins Univ.
770 Press, Baltimore.
- 771 Shackleton LA, Rambaut A, Pybus OG, Holmes EC (2006) JC evolution and its
772 association with human populations. *Journal of Virology*, **80**, 9928-9933.
- 773 Shapiro B, Drummond AJ, Rambaut A, *et al.* (2004) Rise and fall of the
774 beringian steppe bison. *Science*, **306**, 1561-1565.
- 775 Stewart JR, Lister AM, Barnes I, Dalen L (2009) Refugia revisited:
776 individualistic responses of species in space and time. *Proceedings of the*
777 *Royal Society B-Biological Sciences*, **doi:10.1098/rspb.2009.1272**.
- 778 Strasburg JL, Rieseberg LH (2009) How robust are “Isolation with Migration”
779 analyses to violations of the IM model? A simulation study. *Molecular*
780 *Biology and Evolution*, **in press**.
- 781 Strimmer K, Pybus OG (2001) Exploring the demographic history of DNA
782 sequences using the generalized skyline plot. *Molecular Biology and*
783 *Evolution*, **18**, 2298–2305–2298–2305.
- 784 Svensson EM, Anderung C, Baubliene J, *et al.* (2007) Tracing genetic change
785 over time using nuclear SNPs in ancient and modern cattle. *Animal*
786 *Genetics*, **38**, 378-383.
- 787 Tajima F (1983) Evolutionary relationship of DNA sequences in finite
788 populations. *Genetics*, **105**, 437-460.
- 789 Valdiosera CE, Garcia N, Anderung C, *et al.* (2007) Staying out in the cold:
790 glacial refugia and mitochondrial DNA phylogeography in ancient
791 European brown bears. *Molecular Ecology*, **16**, 5140-5148.
- 792 Valdiosera CE, Garcia-Garitagoitia JL, Garcia N, *et al.* (2008) Surprising
793 migration and population size dynamics in ancient Iberian brown
794 bears (*Ursus arctos*). *Proceedings of the National Academy of Sciences of the*
795 *United States of America*, **105**, 5123-5128.
- 796 Wakeley J (2008) *Coalescent Theory: An Introduction* Roberts & Company
797 Publishers.

- 798 Watterson GA (1975) On the number of segregation sites. *Theoret. Popul. Biol.*,
799 7, 256-276.
- 800 Weiss G, von Haeseler A (1998) Inference of population history using a
801 likelihood approach. *Genetics*, **149**, 1539-1546.
- 802 Willerslev E, Cappellini E, Boomsma W, *et al.* (2007) Ancient biomolecules
803 from deep ice cores reveal a forested Southern Greenland. *Science*, **317**,
804 111-114.
- 805 Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97-159.
806
807

808 **Figure 1. The coalescent and summary statistics.** Three coalescent trees are
 809 presented. Mutations are indicated in bold on the resulting sequence alignments
 810 (right). There are $S=4$ segregating sites in all cases. Lineages exclusively ancestral to
 811 ancient DNA samples are marked with a dotted line. (A): isochronous case, average
 812 difference between sequences (total diversity) $\pi=1.8$. (B): moderate heterochrony,
 813 mutation 1 is shared between ancient and modern sequences, mutation 2 is exclusive
 814 to ancient sequences while mutation 3 and 4 are exclusive to modern sequences;
 815 $\pi=2.0$; Nei's net distance: $D_a=0$. (C): large heterochrony, mutations 1, 2 and 3 lead to
 816 fixed differences between modern and ancient sequences while mutation 4 is
 817 exclusive to modern sequences; $\pi=2.2$, $D_a=3$. However, estimates corrected for
 818 heterochrony (Depaulis *et al.* 2009) are: $\pi_h=1.8$ (A), $\pi_h=1.54$, $D_{ah}=-1$ (B) and $\pi_h=1.16$,
 819 $D_{ah}=2.33$ (C).

820
 821 **Figure 2. Skyline plot.** The classical skyline plot analysis uses the reconstructed
 822 genealogy of the sample (a) to obtain estimates of the population size for each time
 823 interval defined by consecutive coalescent events, resulting in a plot shape similar to
 824 the skyline of a city (b). The Bayesian skyline plot (c) implemented in BEAST (Table
 825 1) takes into account the uncertainty within the genealogy of the sample; the final
 826 (smooth) plot represents the posterior probability density for the population size
 827 (typically the median and 95% highest posterior density interval) calculated from the
 828 skyline plots (in grey) for the genealogies sampled with the MCMC.

829
 830 **Figure 3. Likelihood approximation through summary statistics.** A fictitious data
 831 set of ten sequences (five mDNA and five aDNA sampled $0.2 \times N$ generations ago)
 832 containing 20 segregating sites is analysed. The objective of the analyses is to
 833 estimate the parameter $\theta=2N\mu$ of a constant population size model and an IMSM of
 834 mutation. Prior and posterior distributions (estimated with rejection and regression
 835 algorithms) from an approximate Bayesian computation analysis are presented (50%
 836 of simulations rejected from a total of 30 000 simulations; a particularly high rejection
 837 threshold has been chosen for a better illustration of the improvement by the
 838 regression algorithm). Simulations were performed with COMPASS (Table 1) under
 839 the infinite site model and ABC was performed with the R functions of Mark
 840 Beaumont (Table 1). See supplementary files for a script in R to reproduce the
 841 analyses represented in this figure.

Table 1. Software discussed in the main text for the analysis of ancient DNA.

Program	Purpose	Method	Models	Web	Reference
ARLEQUIN	Test heterochrony & population structure	Nested AMOVA, Mantel test	Null model: no effect of time or population structure in genetic diversity	http://cmpg.unibe.ch/software/arlequin3/	Excoffier et al. (2005)
Multivariate Distance Matrix Regression	Test heterochrony & population structure	GAMOVA	Null model: no effect of time or population structure in genetic diversity	http://polymorphism.scripps.edu/~cabney/cgi-bin/mmr.cgi	Nievergelt et al. (2007)
PATH-O-GEN	Test heterochrony	Regression analysis		http://tree.bio.ed.ac.uk/software/pathogen/	n.a.
IBD	Test heterochrony & geographic structure	Partial Mantel test	Isolation by distance and by time	http://www.bio.sdsu.edu/pub/andy/IBD.html	Bohonak (2002)
BEAST	Demographic inference	MCMC	Demography: Flexible (single population) Mutation: Flexible (except SMM) DNA Damage: Yes (Rambaut et al. 2009) Recombination: No	http://beast.bio.ed.ac.uk/	Drummond and Rambaut (2007)
*BEAST	Coalescent-based phylogenetic inference	MCMC	Demography: Population divergence without migration Mutation: Flexible (except SMM) DNA Damage: Yes (Rambaut et al. 2009) Recombination: No	http://beast.bio.ed.ac.uk/	Heled and Drummond (2009)
GENIE	Demographic inference	Classical skyline plot	Demography: Flexible (single population) Recombination: No	http://evolve.zoo.ox.ac.uk/Evolve/Genie.html	Pybus and Rambaut (2002)
R scripts ¹	ABC		Models depend on external simulator	http://www.rubic.rdg.ac.uk/~mab/	Beaumont et al. (2002)
DIYABC	Demographic inference	ABC	Demography: Flexible (except migration) Mutation: SMM ² , K80, HKY, TN ³ (beta version) DNA Damage: No Recombination: No	http://www1.montpellier.inra.fr/CBGP/diyabc/	Cornuet et al. (2008)
BAYESIAN SERIAL SIMCOAL	Coalescent simulation, demographic inference	ABC	Demography: Flexible Mutation: SMM, K80 ³ DNA Damage: No Recombination: No	http://www.stanford.edu/group/hadlylab/ssc/ (includes R scripts for ABC)	Anderson et al. (2005)
COMPASS	Coalescent simulation		Demography: Flexible (single population) Mutation: IMSM ⁴ DNA Damage: No Recombination: No	http://www.egs.uu.se/evbiol/Research/JakobssonLab/compass.html	Jakobsson (2009)
NETRECODON	Coalescent simulation		Demography: Flexible (except admixture)	http://darwin.uvigo.es/software/netrecod	Arenas and

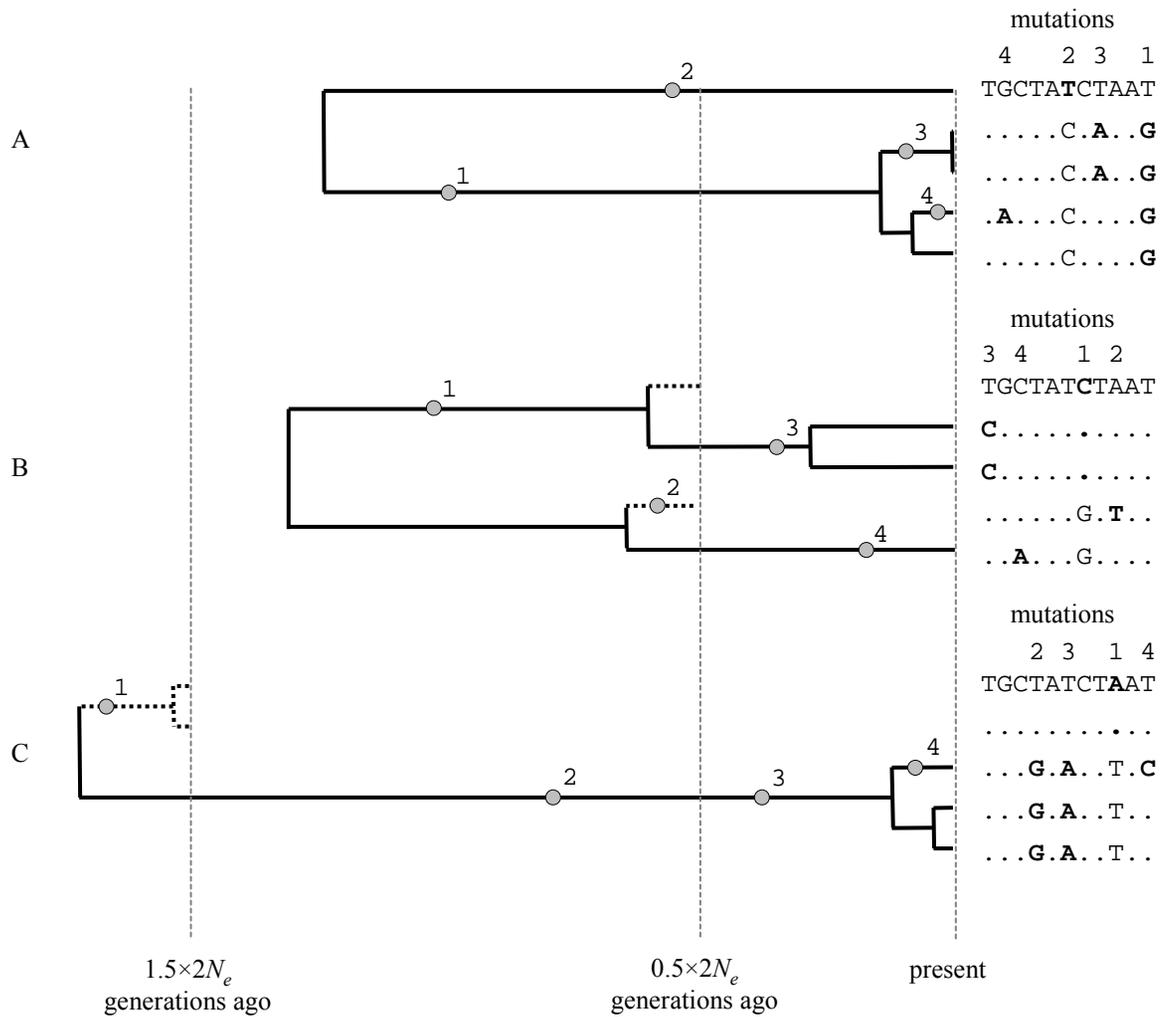
		Mutation: Most DNA models (except IMSM) DNA Damage: No Recombination: Yes	on.html	Posada (2009)
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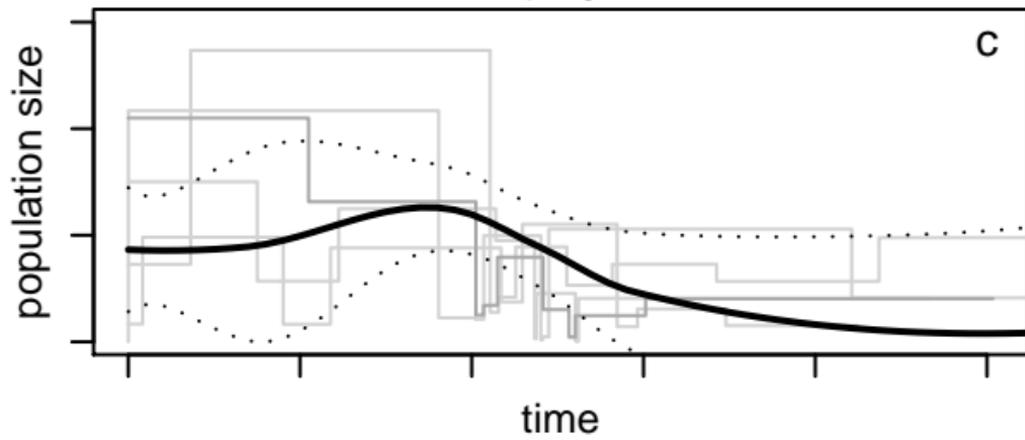
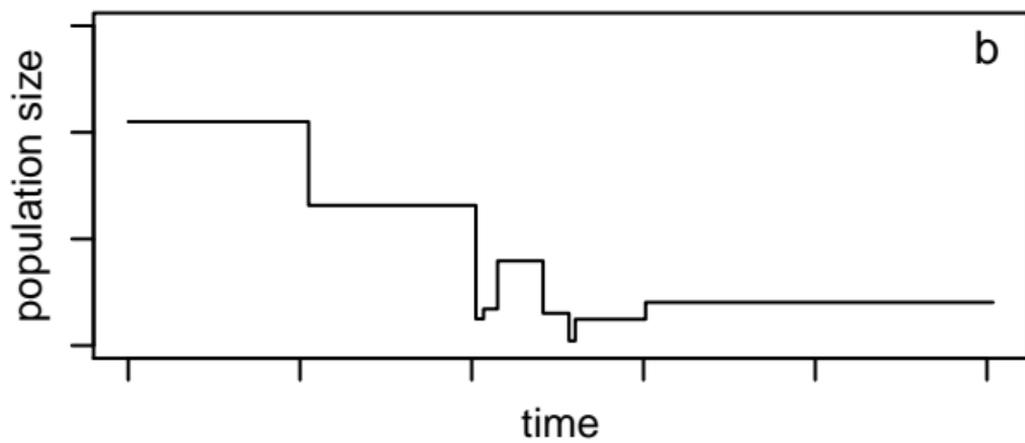
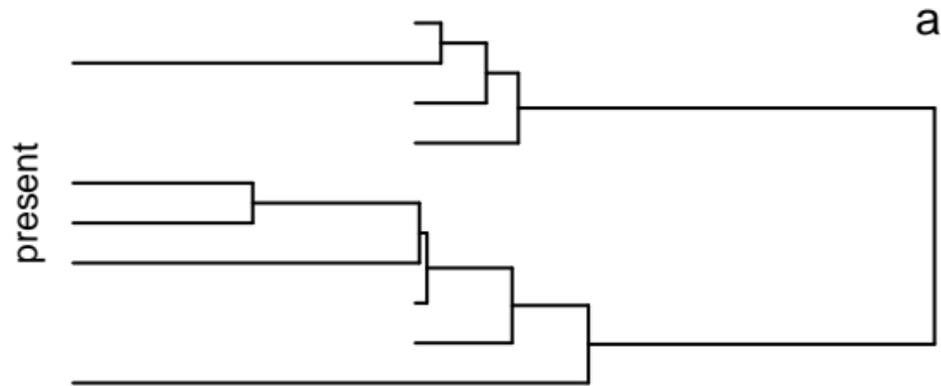
¹ Scripts for the R statistical computing environment (R Development Core Team 2009)

² SMM: stepwise mutation model (for microsatellites)

³ K80: Kimura (1980); HKY: Hasegawa-Kishino-Yano (1985), and TN: Tamura-Nei (1993)

⁴ IMSM: infinitely many site model





probability density

