# Assessing the Maximum Contribution from Ancient Populations

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## Abstract

**Ancestral relationships between populations separated by time represent an often neglected dimension in population genetics, a field which historically has focused on analysis of spatially distributed samples from the same point in time. Models are usually straightforward when two time-separated populations are assumed to be completely isolated from all other populations. However, this is usually an unrealistically stringent assumption when there is gene flow with other populations. Here, we investigate continuity in the presence of gene flow from unknown populations. This setup allows a more nuanced treatment of questions regarding population continuity in terms of "level of contribution" from a particular ancient population to a more recent population. We propose a statistical framework which makes use of a biallelic marker sampled at two different points in time to assess population contribution, and present two different interpretations of the concept. We apply the approach to published data from a prehistoric human population in Scandinavia (Malmström H, Gilbert MTP, Thomas MG, Brandström M, Storå J, Molnar P, Andersen PK, Bendixen C, Holmlund G, Götherström A, et al. 2009. Ancient DNA reveals lack of continuity between Neolithic hunter-gatherers and contemporary Scandinavians. *Curr Biol.* 19:1758–1762) and Pleistocene woolly mammoth (Barnes I, Shapiro B, Lister A, Kuznetsova T, Sher A, Guthrie D, Thomas MG. 2007. Genetic structure and extinction of the woolly mammoth, *Mammuthus primigenius*. *Curr Biol.* 17:1072–1075; Debruyne R, Chu G, King CE, Bos K, Kuch M, Schwarz C, Szpak P, Gröcke DR, Matheus P, Zazula G, et al. 2008. Out of America: ancient DNA evidence for a new world origin of late quaternary woolly mammoths. *Curr Biol.* 18:1320–1326).**

***Key words:*** **ancient DNA, continuity, population genetics.**

## Introduction

The field of ancient DNA (aDNA) research has advanced rapidly during the last few years posing new challenges for population genetics. For example, a number of recent studies have determined mtDNA sequences from prehistoric humans (Ermini et al. 2008; Gilbert et al. 2008; Bramanti et al. 2009; Briggs et al. 2009; Malmström et al. 2009; Krause, Briggs, et al. 2010; Krause, Fu, et al. 2010; Fu et al. 2013)—a task that has been associated with severe problems of authenticity due to the abundance of contaminating modern DNA in excavation and laboratory environments (Cooper and Poinar 2000; Gilbert et al. 2005). Population dynamics of Late Pleistocene mammal populations have been intensively studied using aDNA techniques to understand the impact of climate change and human activities on these species (Hofreiter et al. 2007; Ramakrishnan and Hadly 2009; Hofreiter and Barnes 2010; Lorenzen et al. 2011). A recurring theme in these studies is whether changes in the genetic composition (over time) signals prehistoric turnover events or merely genetic drift. Deviant frequencies of particular mtDNA types (haplogroups) sampled at different timepoints are often taken as evidence for discontinuity between ancient and modern populations. The general rationale in these studies is that a large difference in allele frequency between a modern and an ancient population indicates that the contribution from the ancient to the modern population cannot be too large. However, statistical evaluation of such statements are not always presented (Ramakrishnan and Hadly 2009), or rely on simulation-based model testing (e.g., Valdiosera et al. 2008; Bramanti et al. 2009; Malmström et al. 2009; Ramakrishnan and Hadly 2009; Malmström et al. 2010; Castroviejo-Fisher et al. 2011, but see Serre et al. 2004).

The distribution of the allele frequency difference between an ancestral and a descendent population is a function of accumulated genetic drift between the two populations (we will use the term "population" for time-serial groups of individuals regardless of their relationship). The level of accumulated genetic drift depends, in turn, not only on relatively accessible factors such as number of years between the populations and generation time, but also on more elusive factors such as population size and population structure. Hence, although the age of the ancient sample and the generation time are typically known (to some degree), not much can be inferred from comparing allele frequency differences unless more specific demographic assumptions are made.

The importance of accounting for genetic drift when investigating time structured data was pointed out by Nordborg (1998) in a seminal paper where he reanalyzed mitochondrial data from one Neandertal and 986 modern humans explicitly relying on the coalescent framework to

account for genetic drift. Contrary to the conclusion of Krings et al. (1997), Nordborg (1998) showed that although the data were inconsistent with a single ancient panmictic human–Neandertal population, alternative and more realistic models with a more limited amount of introgression from the Neandertal population could not be ruled out, and a small contribution has indeed been revealed in later studies of autosomal Neandertal DNA (Green et al. 2010).

In fact, rejecting a population as ancestral based on a model that does not allow for any level of introgression from other populations is not very informative: A particular population can be considered ancestral despite the presence of a few immigrants. A more realistic alternative is to investigate the level of contribution from a specified ancient population to a modern population by allowing migration from other populations. Although these populations are in many cases unknown, we can still assess the maximum contribution from a target ancient population that is consistent with the data. This can be done by setting the configuration of the unknown populations to maximize the probability of the observed data.

A common approach to model ancient admixture is to assume an instantaneous admixture "pulse." Under this model, contribution is the fraction of individuals that come from a particular ancient population at the time of the admixture. To illustrate, imagine a target population that existed $t$ units of time before present that instantaneously merged with an unspecified population. The merged population consists of the fraction $c$ of individuals from the target population and the fraction $1 - c$ from the unspecified population, and it evolves for $t$ units of time to become the descendent contemporary population. The interpretation of $c$ for this scenario and in the admixture model framework (Nordborg 1998; Chikhi et al. 2001; Choisy et al. 2004) corresponds to a demographic contribution (fig. 1).

However, since what matters from an evolutionary perspective is the genetic material, any definition of contribution should arguably refer to the fraction of the genetic material in some contemporary population that traces its ancestry to a particular ancient population. Although a demographic contribution of $c$ implies that genetic loci have—on average and assuming an infinite number of independent loci—a proportion $c$ of their ancestry in the target population, the ancestry proportion at any particular locus will typically deviate from $c$. The assumption of a single instantaneous admixture event is moreover often unrealistically restrictive and clearly distinct from models with more than one admixture event or models with continuous gene flow. Also, even if a pulse admixture event did occur, using mtDNA (or markers on sex chromosomes) to study the proportion of genetic material with ancestry in a target ancient population will require information on the sex ratio: if the proportion of females is $f_A$ in the target population and $f_X$ among the incoming individuals, an admixture proportion $c_{mt}$ of mtDNA from the target population implies that the autosomal genome wide proportion of genetic ancestry, $c_{aut}$, in the target population is $c_{mt}f_X/(c_{mt}f_X + (1 - c_{mt})f_A)$ so that $f_A < f_X$ implies $c_{mt} < c_{aut}$ while $f_A > f_X$ implies $c_{mt} > c_{aut}$.

A natural alternative for analyzing nonrecombining chromosomes is thus instead to study genetic contribution, which we define as the proportion of the gene copies in the contemporary population that trace its ancestry back to the older target population (fig. 2). This definition does not, in contrast to demographic contribution, refer to the history of a population but to the ancestry at a specific locus. It represents an alternative that is straightforward to interpret that puts considerably less constraint on the details of the admixture event/process.

In this article, we investigate a method that uses allele frequency changes for assessing an ancient population's genetic and demographic contribution to a more recent population. The approach is based on biallelic genetic data (any data that can be classified into two groups such as SNPs or haplotypes that either belong to, or do not belong to, a

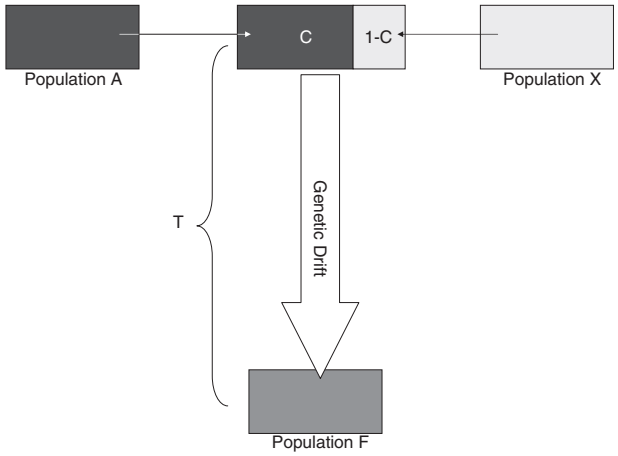


FIG. 1. Model of demographic contribution. A population is created with a fraction $C$ from the ancient population A and the other fraction from unspecified populations (population X). This population is allowed to drift for $T$ units of scaled time to become the younger population F. Note that $T_a = T_s = T$ in this model.

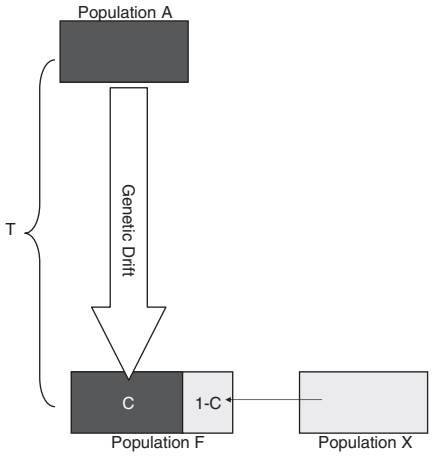


FIG. 2. Model of genetic contribution. The younger population F is split into a fraction $C$ with ancestry from the ancient population A while the other fraction is of unspecified ancestry (population X). The genetic drift between the two populations is represented by $T$. Note that $T_s = T$ while $T_a = 0$ in this model.

specific haplogroup) and assumes that samples from two different points in time have been collected. Utilizing a hypothesis testing framework, the null model with a specified contribution and time (in coalescent units) between the two samples is rejected if, given that this null model is correct, the probability of the observed allele frequency difference between the two samples is less than some cutoff. We show that this approach represents a conservative but still realistic setup such that (in the absence of precise demographic knowledge), if this model is rejected, then most alternative models of ancestral contribution would also be rejected.

We investigate two published data sets with respect to both the maximum genetic and the maximum demographic contribution. Based on a study of mtDNA from prehistoric Scandinavian hunter-gatherers (Malmström et al. 2009), we find that both a major demographic and genetic contribution of mitochondria to the modern Scandinavian population can be rejected. There are however alternative scenarios with a significant contribution from the ancient population which could not be rejected, suggesting that there can be a nontrivial contribution of genetic material from the prehistoric Scandinavians to modern-day Scandinavians. We also investigate a data set of mtDNA from Pleistocene woolly mammoth samples (Barnes et al. 2007; Debruyne et al. 2008).

## Results

Given a difference in allele frequency between two samples, we will formulate an explicit model to quantify the maximum contribution from an ancient population to a more recent population. The approach is similar to that of many previous methods aiming at estimating admixture proportion from two source populations to a hybrid population (Chikhi et al. 2001) as well as estimating effective population size based on temporal allele frequency changes (Nielsen et al. 1998; Berthier et al. 2002; Anderson 2005; Anderson and Slatkin 2007). However, the primary focus of these former studies have been to provide likelihood estimates while our method utilizes a hypothesis testing framework to investigate the possibility of a specific population being ancestral to a more recent population. Moreover, earlier studies have typically assumed that information is obtained from all putative source populations and the possibility of gene flow from unknown populations is not integrated into the methods (but see Wang [2003] for an exception).

### Some Notation

A sample of size $n_F$ from a particular locus in population F ("F" as in "focal") is traced back $T$ units of coalescence time to population A ("A" as in "ancient"). Any population is here defined as the individuals living at a specific time point so that, for example, population F is no longer population F one generation back in time. We assume a biallelic marker with alleles $u$ and "not $u$" ($\bar{u}$) and, without loss of generality, we define $u$ to be the allele that has decreased in frequency from the ancient to the more recent sample ($u$ can thus be either the ancestral or the derived allele). Following the lineages in population F backwards in time to population A, they are assigned to either $u$ or $\bar{u}$ type according to a probability function generated by the observed (aDNA) sample drawn from population A. We denote a sample of size $n_F$ from population F with $m_F$ alleles of type $u$ and $l_F = n_F - m_F$ alleles of type $\bar{u}$ by $\{m_F,l_F\}_\mathbf{F}$ and a sample of size $n_A$ from population A with $m_A$ alleles of type $u$ and $l_A = n_A - m_A$ alleles of type $\bar{u}$ by $\{m_A,l_A\}_\mathbf{A}$. The unknown population(s) will be designated by population $X$ and the frequency of $u$ in population $X$ by $U_X$. We also use $\Delta = m_A/n_A - m_F/n_F$ ($\geq 0$ by definition of $u$) for the observed allele frequency change between the two samples.

### Statistical Setup

Because specifying an alternative model is difficult, we use a classical null-hypothesis testing setup (in the case of an explicit alternative model, a Bayesian approach could have been used). We formulate a null model or null hypothesis and want to test the compatibility of the observed data with this model using a suitable statistic. The null is then rejected if the probability of obtaining the observed value of the statistic—or larger—is below some threshold given the model. The natural choice for such a statistic is, in our case, the allele frequency difference between the two samples as this is the observation that initially suggested that some alternative population may have contributed to population F. There are two pieces of observed data, each corresponding to an alternative choice for setting up the conditioning to calculate a $P$ value: there is the ancient sample configuration $\{m_A,l_A\}_\mathbf{A}$ and the more recent sample configuration $\{m_F,l_F\}_\mathbf{F}$. Hence, besides conditioning on the null, one can condition on either the ancient or the more recent sample configuration (or neither) to calculate a $P$ value. Conditioning on the more recent sample configuration implies that one should calculate the probability of obtaining $\{i,n_A - i\}_\mathbf{A}$ for all $i$ such that $0 \leq i \leq n_A$ and $|i/n_A - m_F/n_F| \geq \Delta$. This has the advantage of being based on the more recent sample, which is typically larger and more trustworthy than the ancient sample. However, a disadvantage is that the mutation that separates allele $u$ from $\bar{u}$ may occur in the time between the two samples and one should (strictly speaking) take the mutation rate into account. As long as the ancient sample is polymorphic, this problem is circumvented by conditioning on the ancient sample. A third alternative corresponds to calculating the probability of observing a frequency difference $\geq \Delta$ without conditioning on either sample but this implies a considerable loss of statistical power. Here, we decide to condition on the ancient sample. In technical terms, we perform a one-tailed test of a change in allele frequency that is greater than or equal to $\Delta$. For reasons outlined in the supplementary material S1 (Supplementary Material online), we also conditioning on that $u$ has decreased in sample frequency between the ancient and the recent sample. Thus, we want to calculate ("con" as in "contribution")

$$f_{\mathrm{con}}(U_X) \equiv \frac{\sum_{i=0}^{m_F} P(\{i, n_F - i\}_{\mathbf{F}} \mid M(t,c,U_X) \wedge \{m_A, l_A\}_{\mathbf{A}})}{\sum_{i=0}^{\left\lfloor n_F \frac{m_A}{n_A} \right\rfloor} P(\{i, n_F - i\}_{\mathbf{F}} \mid M(t,c,U_X) \wedge \{m_A, l_A\}_{\mathbf{A}})} \quad (1)$$

where

$$P(\{m,l\}_{\mathbf{F}} \mid M(t,c,U_X) \wedge \{m_A, l_A\}_{\mathbf{A}}) \quad (2)$$

is a function of $U_X$. Setting $U_X = p_X$ gives the probability of obtaining a modern sample with $m$ $u$ alleles and $l$ $\bar{u}$ alleles conditional on the model $M(T = t, C = c, U_X = p_X)$ and the ancient sample configuration.

If there is information concerning the frequency of $u$ for the unknown populations(s) X, that information can be directly used to integrate over the distribution of $U_X$. An alternative approach would be to treat $U_X$ as a parameter similar to $C$ and $T$ to investigate which combinations of values of $C$, $T$, and $U_X$ that can be rejected. However, $U_X$ can also be seen as a nuisance parameter that we would like to integrate out and one approach would be to find the value of $0 \leq U_X \leq 1$ that maximizes equation (1) to arrive at

$$p_{\mathrm{con}} \equiv \max_{0 \leq U_X \leq 1} f_{\mathrm{con}}(U_X). \quad (3)$$

Note that the maximization of $p_{\mathrm{con}}$ is typically obtained at $U_X = 0$. See supplementary material S1 (Supplementary Material online) and Bayarri and Berger (2000) for a more extensive discussion on the choice of $P$ value.

## The Models

We denote the general null model by $M(t,c,p_X)$, refer to the demographic model by $M_D(t,c,p_X)$ (in which $C$ is interpreted as the demographic contribution) and to the genetic model by $M_G(t,c,p_X)$ (in which $C$ is the genetic contribution). Here, $t$, $c$, and $p_X$ correspond to a particular choice of $T$, $C$, and $U_X$. Figure 1 illustrates $M_D(T,C,U_X)$, where an admixture event occurred $T$ coalescent time units ago in which a new population is created with a fraction $C$ of its gene copies from population A. This newly created population evolves for $T$ coalescent time units to become population F. Figure 2 illustrates the model $M_G(T,C,U_X)$ where a fraction $C$ of the gene copies in population F trace their ancestry back to population A. The probability (2) assuming $M_G(t,c,p_X)$ and $M_D(t,c,p_X)$ are derived in the Materials and Methods section.

## Interpreting $T$

An important population genetic result is that if the mutation process is ignored, the genealogical process in a population with varying size converges to the standard coalescent process regardless of how the population size varies over time (Griffiths and Tavaré 1994; Kaj and Krone 2003). Assuming that the mutation that separates the two alleles is older than population A, the convergence to the standard coalescent process implies that population size changes during the time between population A and population F do not affect our calculations as long as we condition on the (correctly scaled) time $T$.

$T$ is measured in units of coalescence time which, in calendar time, corresponds to $T$ multiplied by both $N_e$ (the effective population size) and the number of years per generation. We use "$N_e$" for the effective number of chromosomes so that time is scaled in $N_e$ generations, not $2N_e$ generations as is the case for autosomal markers. $T$ depends on the number of generations, $G$, between population F, and population A, and the size $N_i$ of the population at generation $i$ (more specifically, the inverse of the strength of genetic drift at generation $i$) before present, through

$$T = \sum_{i=1}^{G} \frac{1}{N_i}$$

(Griffiths and Tavaré 1994). If $G$ is assumed to be between $G_{\min}$ and $G_{\max}$ and we have some bounds on $N_i$, $N_{\min} \leq N_i \leq N_{\max}$ for $1 \leq i \leq G_{\max}$, the bounds on $T$ become

$$\frac{G_{\min}}{N_{\max}} \leq T \leq \frac{G_{\max}}{N_{\min}}. \quad (4)$$

## Evaluating the Robustness of the Approach Using Simulations

We evaluate the robustness of our approach using the simulation procedure described in the Materials and Methods section. First, we simulate to check our analytical framework. We simulate genetic data under a model of complete population continuity ($C = 1$) and find that the analytical formulas agree with both COMPASS and (the modified) *ms* (fig. 3). Second, we simulate a model where the contemporary population descended from an admixture event between two ancient populations ($C < 1$). At the time of admixture, descendant lineages are assigned to the two populations with probability $C$ and $(1 - C)$, respectively, and we keep only those simulations that produce the particular configuration for the ancient sample which we condition on. To mimic the analytical approach, we conditioned on observing zero $u$ alleles in a sample ($n = 60$) from the alternative ancient population (population $X$) and we found close concordance to the analytical computation (fig. 3).

To mimic the data in the example of Neolithic Europe (Malmström et al. 2009), we conditioned on observing 11 $u$ alleles in an ancient sample of size 19 and counted the frequency of the variant in a sample from the contemporary population (to speed up the simulation procedure, we use a sample of size 20 for the contemporary sample instead of 290 as in Malmström et al. [2009]) and set the contribution $C$ to 0.8. For this example, we investigated the effect of two potential violations of model $M_D$: 1) If the time of the admixture event and the age of the ancient sample are not the same, and 2) if the age of the ancient samples are different from each other. More specifically, for case 1, we investigated scenarios where $T_a$ equalled 0.05, 0.1, 0.15, and 0.2, and $T_s$ remained fixed at 0.2. For case 2, we considered a scenario

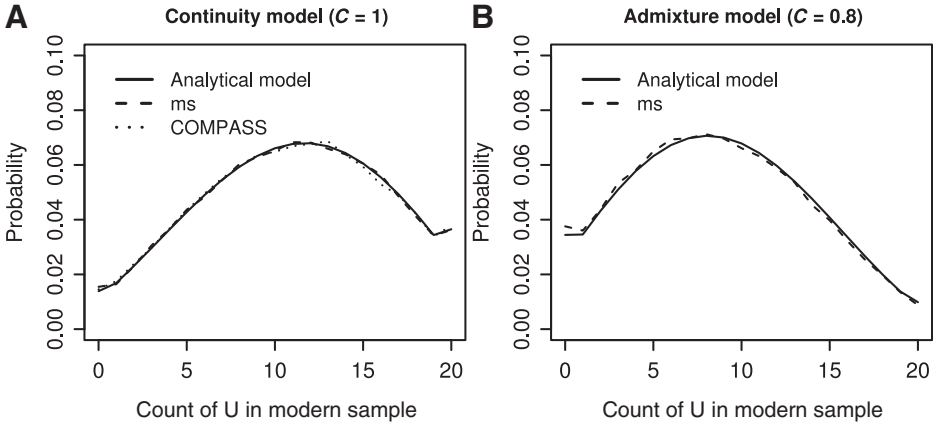


FIG. 3. Comparison of the analytical approach to coalescent simulations. Allele frequency spectrum in the modern sample obtained for (*A*) $C = 1$ (continuity model) and (*B*) $C = 0.8$ (admixture model) are displayed.

where the ages of the $n = 19$ ancient samples were symmetrically dispersed in time around $T_a = 0.2$, and investigate the resulting allele frequency spectrum as a function of the root mean square difference to the mean $D_T$,

$$D_T = \sqrt{\sum_{j=1}^{n} (T_a - T_j)^2}$$

where $T_j$ is the age of sample $j$. For comparison, we simulate data under model $M_D$ setting the contribution $C$ to 0.8, and setting both the ancient sampling time $T_s$ and time of admixture $T_a$ to 0.2.

Looking backwards in time, the analytical approach assumes that the admixture event occurs immediately before the time of the ancient sample for model $M_D$. When decreasing the time of the admixture event ($T_a < T_s$), we find that the probability of observing a derived allele frequency less than 5% in the modern sample decreases (fig. 4). In the second simulation, when the ages of the ancient samples ($T_s$) are symmetrically distributed in time (fig. 5), the probability of observing an allele frequency less than 5% in the modern sample also decreases. This result implies that the analytical method is robust for two features which are common for many aDNA studies, variation in age among the ancient samples and the ancient samples being older than the demographic event of interest. We conclude that (at least under the particular cases simulated) inference under our analytical approach is robust (since the probability of the data given for our model is greater than under the alternative models) to both the admixture event being more recent than the age of the ancient sample and to the ancient sample being spread over time.

## Population Continuity in Neolithic Europe

Two studies, Bramanti et al. (2009) and Malmström et al. (2009), used ancient mitochondrial DNA to address one of the most long-standing questions in human evolutionary genetics: The extent to which population replacement was associated with the Neolithization of Europe (Cavalli-Sforza et al. 1993; Jobling et al. 2004; Currat and Excoffier 2005; Skoglund et al. 2012). Both studies (Bramanti et al. 2009; Malmström et al. 2009) use simulations to investigate whether a prehistoric hunter-gatherer population can be the ancestral population of a more recent population. If the prehistoric hunter-gatherer population can be the ancestral population of the more recent (farmer) population, this finding would indicate that agriculture was introduced to Europe as transmission of ideas rather than a replacement of peoples. To investigate whether the prehistoric hunter-gatherer population can be ancestral to the more recent population, Bramanti et al. (2009) and Malmström et al. (2009) compared levels of genetic differentiation ($F_{ST}$) for simulated data generated under particular population-genetic models and $F_{ST}$ for the observed empirical data. In both studies, the authors conclude that (complete) continuity can be rejected and that this result indicates that the prehistoric hunter-gatherer populations were replaced by people who brought farming practices to Europe (Bramanti et al. 2009; Malmström et al. 2009; Rowley-Conwy 2009). However, whether the inferred replacement affected the majority of the hunter-gatherer population or just a small fraction was not investigated.

Using our approach, we can quantify the maximum level of contribution from the prehistoric Scandinavian hunter-gatherer population to present day Scandinavians that is compatible with the observed allele frequency difference (Malmström et al. 2009). We focus on the observation that 11 of 19 prehistoric Scandinavian hunter-gatherers belonged to a particular mitochondrial haplogroup, the monophyletic (and derived) "U"/"K" clade, and that in a sample of 290 modern Scandinavians, only 26 individuals carry haplogroups "U" or "K." The age of the fossils (from the prehistoric Scandinavian hunter-gatherer population) are between 4,000 and 4,800 years old (Malmström et al. 2009) and if we assume a generation time of 25 years, the chronological time corresponds to between 160 and 192 generations. The range of 2,000 to 5,000 for the mitochondrial effective population size $N_e$ corresponds to $0.032 \leq T \leq 0.096$ time units (see eq. 4). For the extreme upper bound on the scaled time ($T = 0.096$), we can reject ($p_{con} < 0.05$) a demographic contribution greater than 61% and a genetic (mtDNA)

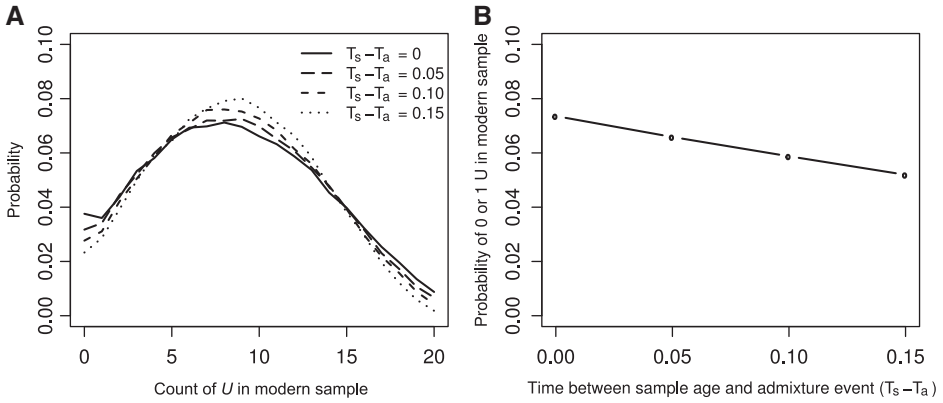


**Fig. 4.** The effect of difference in time between the admixture event and the age of ancient samples. (*A*) Expected derived allele frequency spectrum in the modern sample given 11 (derived) *u* alleles out of 19 in the ancient sample for different assumptions on $T_s - T_a$. (*B*) The probability of allele frequency $\leq 5$ % (0 or 1 copies of the *u* allele) in the modern sample decreases with $T_s - T_a$.

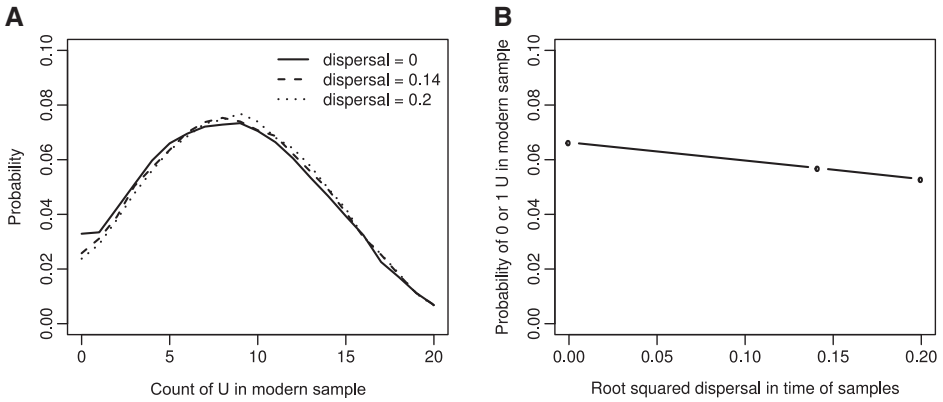


**Fig. 5.** The effect of ancient samples being symmetrically dispersed in time. (*A*) Expected allele frequency spectrum of derived allele *u* in the modern sample given 11 *u* alleles out of 19 in the ancient sample for different assumptions on the degree of time dispersal $D_T$. (*B*) The probability of allele frequency $\leq 5$ % (0 or 1 copies of the *u* allele) in the modern sample decreases with $D_T$.

contribution greater than 40% from the prehistoric hunter-gatherer population to modern Scandinavians (figs. 6 and 7). If we assume that $N_e = 3{,}400$ and samples being 4,400 years old, we get $T = 0.052$ and can reject ($p_{con} < 0.05$) a demographic contribution of $C \geq 0.49$ and a genetic contribution of $C \geq 0.34$ to modern Scandinavians. Thus, both a range of values for *T* and the point estimate used in Malmström et al. (2009) suggest that a substantial proportion of the modern Scandinavian population does not trace back to the sampled prehistoric hunter-gatherer population.

To summarize, for the range of realistic values of *T*, we show that a demographic contribution larger than 61% can be rejected as well as that more than 40% of the mtDNA in the modern Scandinavian population has an origin in the prehistoric Scandinavian hunter-gatherer population. Our results thus show, similar to the conclusion of Malmström et al. (2009), that the prehistoric Scandinavian hunter-gatherer population cannot be claimed to be the sole ancestral population to present day Scandinavians. Conversely, we find that up to 40% of the present day Scandinavian mitochondrial gene pool could not be excluded to have a prehistoric Scandinavian hunter–gatherer origin, suggesting that a sizable fraction of the genomes of modern Scandinavians could have a prehistoric Scandinavian hunter–gatherer ancestry. Compared with the conclusions drawn in Malmström et al. (2009), our method provides additional insight as it pinpoints alternative interpretations that would not be inconsistent with the data.

## Population Continuity in Siberian Woolly Mammoths

In Siberian woolly mammoths, a particular mtDNA haplogroup has been observed to be lost over time, and the haplogroup is absent from two younger samples (Barnes et al. 2007; Debruyne et al. 2008). Debruyne et al. (2008) observed that in a sample of 47 individuals older than 44,000 years, 11 were assigned to Clade A, while the frequency of Clade A was 0/61 in samples between 44,000 and 22,000 years ago. These observations suggest that a drastic population turnover occurred somewhere between 44,000 and 22,000 years ago (Debruyne et al. 2008). We can calculate the scaled time interval using equation (4) if we have a range of possible (effective) population sizes as well as a time difference in generations between the samples. A problem here is that dates of the samples in both the younger and the older

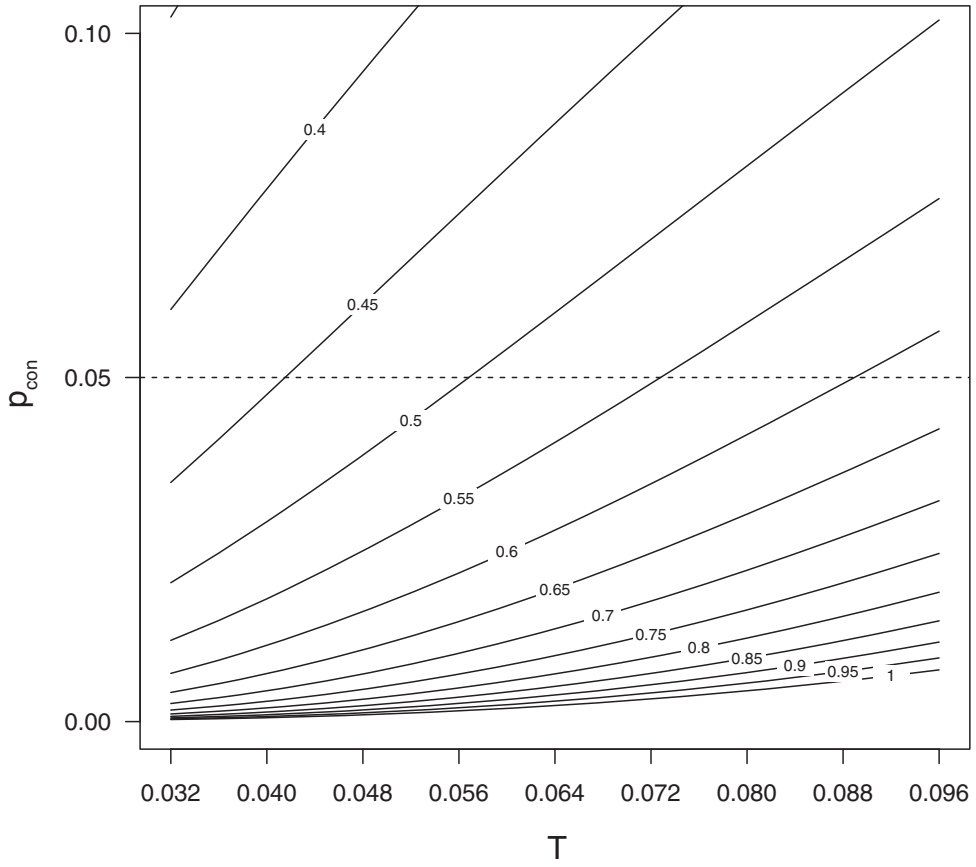


**Fig. 6.** $p_{con}$ values under the null models of demographic contribution for the observed frequency difference between current-day Scandinavians and prehistoric Scandinavian hunter-gatherers over $0.032 \leq T \leq 0.096$. The black dotted horizontal line represents the rejection level ($p_{con} = 0.05$). $C$ from 0.4,0.45, $\cdots$ ,1 are shown in the figure. The $1/x$ prior was used for the frequency of the derived allele $u$ in population A as this was the more conservative choice for this example.

samples are spread out and that many of the dates in the older sample are lower bounds. However, relying on our simulation study, we use the mean of the ages in the two samples. Using the published age estimates in Debruyne et al. (2008), the mean and median age of the samples in the younger sample is approximately 35,000 years old (mean = 35,130, median = 35,055, standard deviation [SD] = 5,468) while the mean and median for the older sample is around 50,000 years old (mean = 49,915, median = 49,709, SD = 5,063) giving an average time difference of 15,000 years between the samples. Here, the lower bounds in the older sample are assumed to be point estimates and we note that 15,000 years is probably an underestimate. Assuming that the generation time of woolly mammoths is approximately 15 years (Sukumar 1989, p. 179) leads to an average number of 1,000 generations between the samples. A recent estimate based on nuclear microsatellite data (Nyström et al. 2012) gives a 95% confidence interval for $N_e$ from 5,000 to 23,400 which translates to 2,500 — 11,700 effective number of mitochondria. Using equation (4), we arrive at the range $0.09 < T < 0.4$ of scaled time between the two groups of samples. Although it is possible to reject a more than 50% genetic contribution at $T = 0.09$, the power to reject demographic contribution is considerably lower (only a more than 90% contribution can be rejected at $T = 0.09$). Moreover, $T = 0.09$ is the extreme lower bound and for only a slightly larger $T$ ($T > 0.1$) any level of contribution (genetic or demographic) from the older population to the younger population is compatible with the observed allele frequency difference (figs. 8 and 9). Hence, unless these two time-structured groups of samples are assumed to be very close in time and/or the populations have very large effective population sizes (both unlikely assumptions), so that $T < 0.1$, the observed allele frequency difference contains little information about the demographic history of woolly mammoths.

## Discussion

We have described an approach for interpreting time-structured population genetic data. Our approach is specifically geared toward dealing with cases where the allele frequency in a sample from an ancient population is very different from the frequency in a sample from a modern population. Such observations are generally considered as evidence for population discontinuity. By explicitly allowing inflow of genetic material from other (unknown) populations in our model, the question of population discontinuity can be rephrased and tested in a more meaningful way. Our method provides an upper bound on the contribution from the sampled ancient population to the modern population. We show that this upper bound is robust to the two most obvious violations

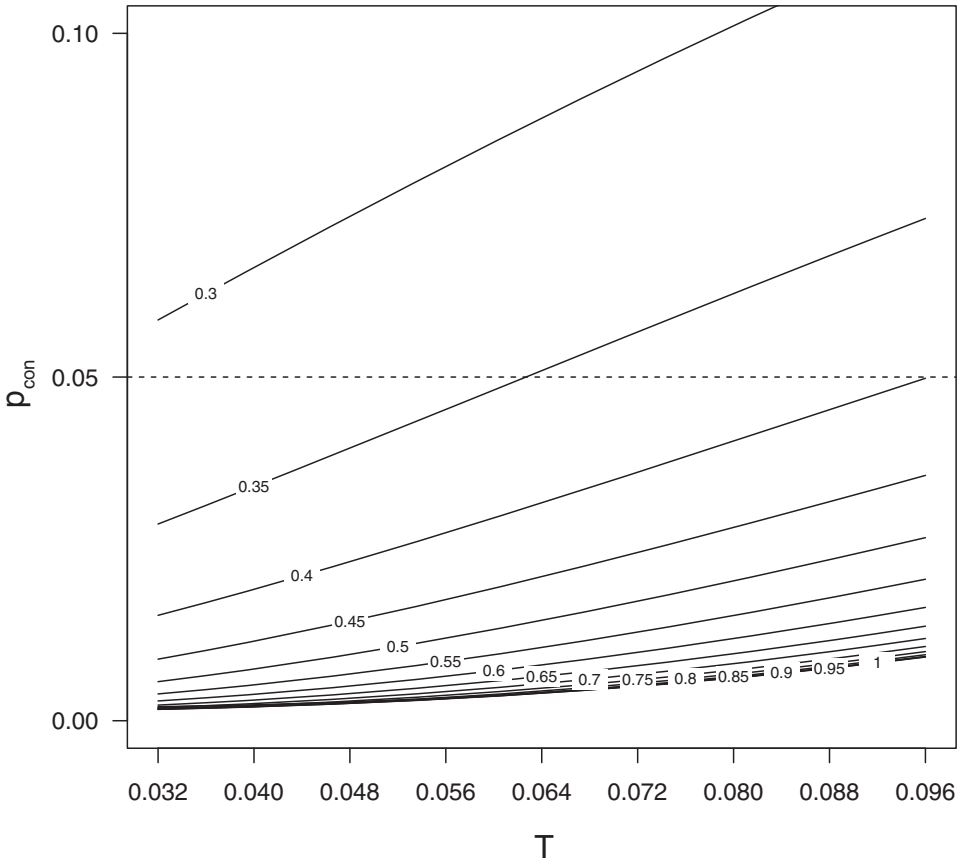


**Fig. 7.** $p_{con}$ values under the null models of genetic contribution for the observed frequency difference between current-day Scandinavians and prehistoric Scandinavian hunter-gatherers over $0.032 \leq T \leq 0.096$. The black dotted horizontal line represents the rejection level ($p_{con} = 0.05$). $C$ from 0.3,0.35, $\cdots$,1 are shown in the figure. The $1/x$ prior was used for the frequency of the derived allele $u$ in population A as this was the more conservative choice for this example.

of the model assumptions, namely that the admixture event is more recent than the age of the older sample and that the older sample is spread out in time (fig. 4*B* and 5*B*, respectively). Therefore, rejecting the simple pulse-admixture model implies the rejection of the most relevant more realistic models.

We assume strict neutrality of the investigated locus and although some would argue that, especially for mitochondrial data, this is not a valid assumption (Hudson and Turelli 2003) and, similar to other studies that assume neutrality for particular loci to infer demographic history, we acknowledge that selection can impact our test.

We apply our method on two different data sets. By first demarcating a range of possible values of (scaled) time between the two samples in Malmström et al. (2009), we show that a contribution larger than 61% (demographic contribution) from the sampled prehistoric population to the contemporary Scandinavian population can be rejected. Furthermore, at most 40% of mitochondria in the present Scandinavian population has ancestry in the sampled ancient population (genetic contribution). This example is a good illustration of that biallelic, nonrecombining markers—with limited amount of information concerning demographic history—can provide useful information as long as it is possible to narrow down the range of realistic values of $T$.

The range of scaled time between the two samples of woolly mammoth (Barnes et al. 2007; Debruyne et al. 2008; Nyström et al. 2012) was not of much help as the ages of the sampled bones were too spread out in time. This clearly illustrates the connection between external information and power of our method. The woolly mammoth example also illustrates how $T$ and $C$ are connected as there is not much room even for a minor contribution if the scaled time that separate the two populations is close to zero but any level of genetic and demographic contribution is possible given $T > 0.1$. For the woolly mammoth example, this means that the mtDNA data and the observed allele frequency difference does not provide much information on the demographic events in the time period. Perhaps a finer binning of the samples could improve the inferences or, as suggested by Debruyne et al. (2008), the older sample could be considered as drawn from two populations—an American/Alaskan and an Asian/Siberian.

There are many alternative ways of how to construct the null models. One could for instance condition on the more recent sample from population F. There are advantages to this change of perspective, perhaps the most obvious being that in many cases, markers are chosen because they are polymorphic in the modern population making the conditioning on the younger of the two samples more natural.

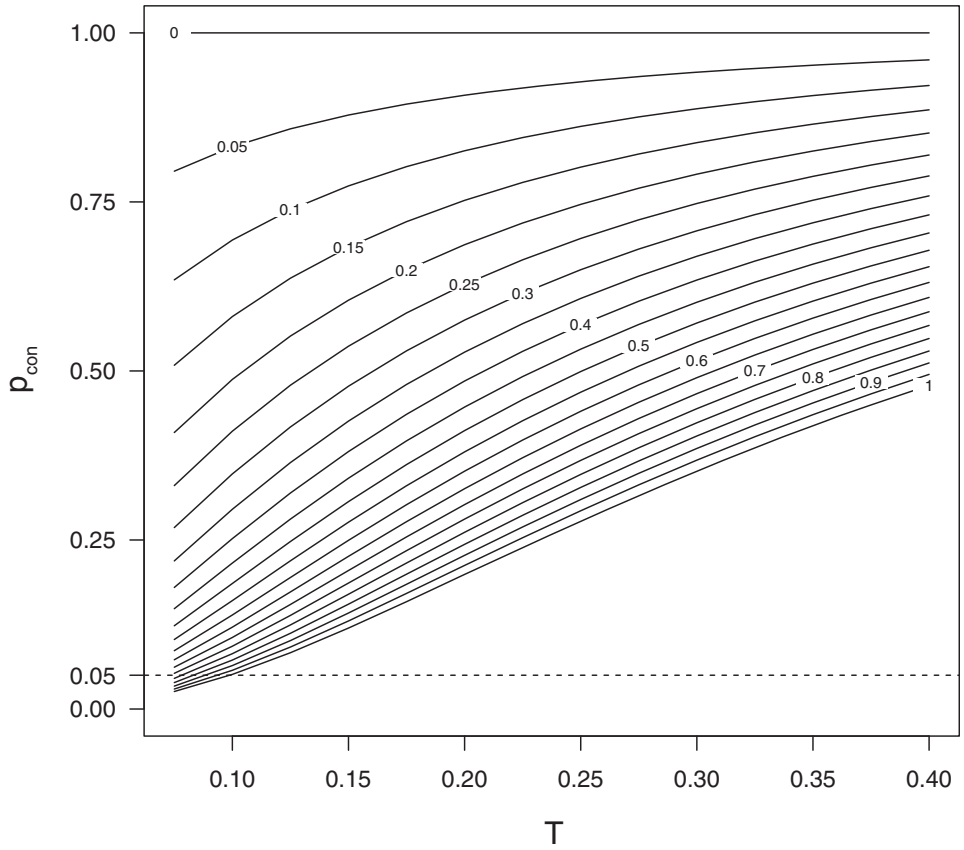


**Fig. 8.** $p_{con}$ values under the null models of demographic contribution for the observed frequency difference between the older and the more recent Siberian woolly mammoth populations over $0.09 \leq T \leq 0.4$. $C$ from 0, 0.05, $\cdots$, 1 are shown in the figure. The uniform prior was used for the frequency of the derived allele $u$ in population A as this was the more conservative choice for this example.

However, the age of the mutation that separates the two alleles becomes problematic if we condition on the sample from population F because we need to directly model the mutation process (which makes the setup more complicated). Perhaps, simply ignoring the possibility that the mutation arose during the time interval between population A and population F is a good approximation but its validity will depend on the particular situation (the same problem arise in our setup if the ancient sample does not contain any derived alleles).

We discuss some technical subtleties that are inherent in setting up a statistical framework for testing contribution levels based on a biallelic single locus. We point out that for model $M_G$ the interpretation of "a contribution $C$ from population A to population F" is the percentage of genetic material in population F that traces back to population A, and this percentage is valuable information for the particular locus. On the other hand, the standard admixture model (the demographic contribution model) is shown to be conservative in that small alterations of it will make it easier to reject suggesting that relying on the admixture model may simply be a conservative choice in that most alternative demographic models with the same (expected) amount of genetic material tracing back to population A may be easier to reject.

As is evident in figure 9 and as investigated in the supplementary material S1 (Supplementary Material online), $p_{con}$ is not well behaved for large values of $T$ under the genetic contribution model. This property is likely related to the fact that we perform a one-sided test of frequency change and if $T$ is large, $p_{con}$ loses power. However, our setup is geared toward low to medium values of $T$.

Although some scenarios may give inconclusive results such as illustrated by the woolly mammoth example, which reflects the low information content in the data combined with large uncertainties of model assumptions, the test is remarkably powerful in other cases. This observation is especially true considering that the test relies on a single marker. The power is clearly related to how much the possible range of scaled time can be narrowed down. This is likely to be at least partly explained by the peaky distribution of the function $g(k; n,t)$ (Jakobsson and Rosenberg 2007; Maruvka et al. 2011) implying that a large amount of information concerning values for $t$ translates into a large amount of information concerning the number of ancestors in the older population. This idea also let us speed up our calculations considerably with minimal loss of accuracy as explained in the supplementary material S1 (Supplementary Material online). The formulas utilized here involve heavy computing and we used multiple precision C++ libraries (MPFR [www.mpfr.org] and GMP [gmplib.org]) to calculate the necessary

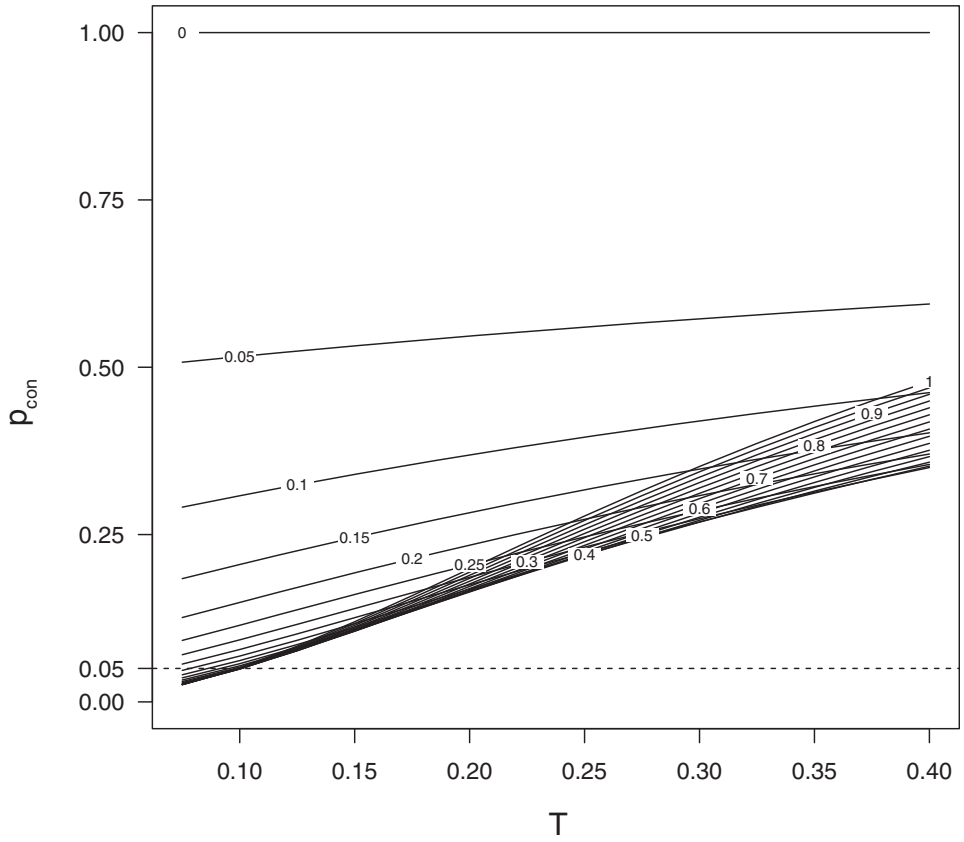


**Fig. 9.** $p_{con}$ values under the null models of genetic contribution for the observed frequency difference between the older and the more recent Siberian woolly mammoth populations over $0.09 \leq T \leq 0.4$. $C$ from $0,\ 0.05, \cdots, 1$ are shown in the figure. The uniform prior was used for the frequency of the derived allele $u$ in population A as this was the more conservative choice for this example.

probabilities (see supplementary material S1, Supplementary Material online, which also outlines a simpler simulation procedure to approximate $p_{con}$). Hypothesis testing is one approach to the problem of detecting population turnover and in the case of no candidate alternative population, it may be the best option. Several studies have previously addressed the situation when there are two (or more) alternative known populations that are potential ancestral populations to a more recent population (e.g., as in Bramanti et al. 2009). Cabana et al. (2008) relied on a hypothesis testing framework and simulations of relatively complex demographic models while most studies are based on the admixture model framework with the question shifted toward estimating the contribution of each population (Bertorelle and Excoffier 1998; Chikhi et al. 2001; Wang 2003; Choisy et al. 2004; Sousa et al. 2009; Finger et al. 2011).

To conclude, our approach advances the potential interpretation of aDNA data beyond the question of continuity or discontinuity to a more nuanced situation for assessing the contribution from an ancient population to a more recent population. The problem described here deals with single locus biallelic data but it can likely be extended it to a multiloci case. Furthermore, as multiloci data from multiple ancient individuals become available (Skoglund et al. 2012), much of the fundamental theory that we use for hypothesis testing can be used for designing approaches to estimate the contribution from ancient to more recent populations.

## Materials and Methods

### Assuming Absolute Continuity

If $C = 1$, population A is the unique ancestral population to population F (irrespective of model $M_D$ or $M_G$) such that the probability (2) equals

$$\Phi(m,l;\, m_A,l_A,t) \equiv \sum_{\alpha=0}^{m}\sum_{\beta=0}^{l} h(m,l;\,\alpha,\beta)s(\alpha,\beta;\, m_A,l_A)g(\alpha+\beta;\, m+l,t) \quad (5)$$

(an adaptation of a result in Chikhi et al. 2001), where

a) $g(k;\, n,t)$: the probability of $n$ genes having $k$ ancestors $t$ units of coalescent time ago,
b) $s(\alpha,\beta;\, m,l)$: the probability to draw $\alpha$ $u$ alleles in a sample of size $\alpha+\beta$ conditioning on a previous sample with $m$ $u$ alleles and $l$ $\bar{u}$ alleles, and
c) $h(m,l;\,\alpha,\beta)$: the probability that there are $m$ lineages of type $u$ when there are $m + l$ lineages given that $\alpha$ lineages are of type $u$ when there are $\alpha+\beta$ lineages ($\alpha+\beta \leq m+l$).

These functions are known and they are given in the appendix. We discuss below the problem of choosing a prior for the distribution of the allele frequency in population A, which is needed for computing $s(\alpha,\beta; m,l)$. Note that $\Phi(m_F,l_F; m_A,l_A,T=0) = s(m_F,l_F; m_A,l_A)$.

## Genetic Contribution

When $C$ is interpreted as genetic contribution, a fraction $C$ of the gene copies (at a single nonrecombing locus) in population F trace back to population A (fig. 2). A sample of size $n_F$ thus contains $0 \leq k \leq n_F$ gene copies that trace back to population A with probability $\binom{n_F}{k} C^k (1-C)^{n_F-k}$. The number of $u$ alleles among the $n_F - k$ gene copies that do not trace back to population A is binomially distributed with parameters $n_F - k$ and $p_X$. Consequently, the probability (2) is

$$\sum_{\alpha=0}^{m}\sum_{\beta=0}^{l} Bin(\alpha+\beta; m+l,c)\Phi(\alpha,\beta; m_A,l_A,t) \times Bin(m-\alpha; m+l-\alpha-\beta,p_X) \quad (6)$$

where $m + l = n_F$ and $Bin(k; n,x) \equiv \binom{n}{k} x^k (1-x)^{n-k}$.

## Demographic Contribution

When $C$ is interpreted as demographic contribution, at time $T$ backwards in time a proportion $C$ from population A and a proportion $1 - C$ from population $X$ merges and creates an admixed population that evolves for $T$ time units to become the present population F (fig. 1). Thus, instead of following a reduced genealogy as is done for genetic contribution, the whole genealogy with $n_F$ external branches is followed backwards $T$ units of coalescent time. At time $T$ when the genealogy enters population A, the situation is exactly (except that $m + l$ is not necessarily equal to $n_F$), the same as when $C$ is interpreted as genetic contribution for $T = 0$. Thus (since $\Phi(i,j; m_A,l_A,0) = s(i,j; m_A,l_A)$), by defining

$$\zeta(\alpha,\beta; m_A,l_A,c,p_X) \equiv \sum_{i=0}^{\alpha}\sum_{j=0}^{\beta} Bin(i+j; \alpha+\beta,c) \times s(i,j; m_A,l_A)Bin(\alpha-i; \alpha+\beta-i-j,p_X) \quad (7)$$

(the probability to obtain $\alpha$ $u$ alleles in a sample of size $\alpha + \beta$) and by summing over the number of ancestors to the sample at time $T = t$, equation (2) can be written as

$$\sum_{\alpha=0}^{m}\sum_{\beta=0}^{l} h(m,l; \alpha,\beta)\zeta(\alpha,\beta; m_A,l_A,c,p_X)g(\alpha+\beta; n_F,t). \quad (8)$$

## The Frequency Distribution of $u$ in the Sampled Ancient Population

Generating a distribution for the frequency of an allele in a population based on a sample from this population requires that a prior distribution for the allele frequency is specified. Among priors that can be argued to reflect our beliefs (before the sampling), we choose the prior that gives the greatest probability of the observation. One possible prior is the uniform prior as it corresponds to "no information" (note that admixture estimates—which is not our aim—can be biased if a uniform prior is used and alternative priors have been suggested to cope with this problem [Wang 2003; Choisy et al. 2004; Anderson 2005]). Alternatively, if it is known whether $u$ is derived or ancestral, one might use a density $1/x$ for the frequency of the derived allele (e.g., Griffiths 2003), which assumes a standard Wright–Fisher model with constant population size and no population structure. We find that the choice of prior (out of these two) is of minor consequence in the investigated cases.

## Simulations to Evaluate the Robustness of the Approach

To evaluate the performance and robustness of our analytical approach, we conduct simulations based on the model with a demographic interpretation of contribution, $M_D$, and simulations based on models where some assumption in model $M_D$ is violated. In model $M_D$, it is assumed that $T = T_a = T_s$ where $T_a$ is the time of admixture and $T_s$ the time of sampling.

We generated data for one variable site using an algorithm which allows time-serial samples to be drawn from a single population under a coalescent model implemented in the software COMPASS (Jakobsson 2009). To assess the effect of admixture between populations, we used the simulation software *ms* (Hudson 2002), which allows an arbitrary number of structured populations and migration schemes, but does not allow samples from historical time points. To accommodate historical samples, we modified the input and output of *ms* using the following algorithm:

1) For an ancient sample of size $L$, $L$ isolated subpopulations are created with one single lineage sampled from each subpopulation.
2) At the desired sample time, $T_s$, of the ancient sample, the $L$ subpopulations are joined with the population from which they are to be drawn.
3) From the gene tree output of *ms*, we subtract $T_s$ from the external branch of each ancient sample and generate segregating sites on the resulting trimmed genealogy with probability proportional to branch length (Hudson 1990).

We approximated the probability of different allele frequencies in the ancient and the contemporary populations by conditioning on a specific sample frequency in the ancient population and retaining those simulations where we observe exactly the same configuration (e.g., Malmström et al. 2010) until we have 100,000 simulated data sets. From this set, we record the frequency of the *derived* allele in the contemporary population, and we obtain an approximation of the expected derived allele frequency spectrum in the contemporary sample. From this distribution, we get an (approximate) probability of observing a frequency equal to or more extreme than the observed frequency in an empirical sample.

### Software

A computer program package "MaxCon" that implements these ideas is available at www.ebc.uu.se/Jakobsson/software/MaxCon/ (last accessed February 18, 2014).

## Supplementary Material

Supplementary material S1 is available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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