Factors influencing ascertainment bias of microsatellite allele sizes: impact on estimates of mutation rates

Biao Li§1 and Marek Kimmeľ§,†*

§Departments of Statistics and Bioengineering, Rice University, Houston, TX 77005, USA
†Systems Engineering Group, Silesian University of Technology, Gliwice, Poland

* These authors contribute equally to this work.
Keywords: microsatellite ascertainment bias, mutation rate, demography, coalescence, forward-time simulation

1Corresponding author: Biao Li, 6100 Main St. MS-138, Houston, TX 77005. E-mail: li.biao@rice.edu
Abstract

Microsatellite loci play an important role as markers for identification, disease gene mapping and evolutionary studies. Mutation rate, which is of fundamental importance, can be obtained from interspecies comparisons, which however are subject to ascertainment bias. This bias arises for example when a locus is selected based on its large allele size in one species (cognate species 1), in which it is first discovered. This bias is reflected in average allele length in any non-cognate species 2 being smaller than that in species 1. This phenomenon was observed in various pairs of species, including comparisons of allele sizes in Human and Chimpanzee. Various mechanisms were proposed to explain observed differences in mean allele lengths between two species. Here, we examine the framework of a single-step asymmetric and unrestricted stepwise mutation model with genetic drift. Analysis is based on coalescent theory. Analytical results are confirmed by simulations using the simuPOP software. The mechanism of ascertainment bias in this model is a tighter correlation of allele sizes within a cognate species 1 than of allele sizes in two different species 1 and 2. We present computations of the expected average allele size difference, given the mutation rate, population sizes of species 1 and 2, time of separation of species 1 and 2, and the age of the allele. We show that when the past demographic histories of the cognate and non-cognate taxa are different, the rate and directionality of mutations impact the allele sizes in the two taxa differently from the simple effect of ascertainment bias. This effect may exaggerate or reverse the effect of difference in mutation rates. We re-analyze literature data, which indicate that despite the bias, the microsatellite mutation rate estimate in the ancestral population is consistently greater than that in either Human or Chimpanzee and the mutation rate estimate in Human exceeds or equals that in Chimpanzee with the rate of allele length expansion in Human being greater than that in Chimpanzee. We also demonstrate that population bottlenecks and expansions in the recent human history have little impact on our conclusions.
Introduction

Ascertainment bias in population genetics is usually studied in two contexts. One of them is discovery of polymorphic loci and it is best illustrated by the example of SNPs (single-nucleotide polymorphisms). As demonstrated in a number of papers, taking into account the ascertainment scheme is a very important aspect of SNP data analysis. For example, Polanski and Kimmel (2003) derived expressions for modeling the way in which ascertainment modified SNP sampling frequencies and distorted inferences concerning the mutation rate. A more recent paper (Albrechtsen et al. 2010) considers chip-based high-throughput genotyping, which has facilitated genome-wide studies of genetic diversity. Many studies have utilized these large data sets to make inferences about the demographic history of human populations. However, again, the single nucleotide polymorphism (SNP) chip data suffer from ascertainment biases caused by the SNP discovery process in which a small number of individuals from selected populations are used as discovery panels. Albrechtsen et al. (2010) demonstrate that the ascertainment bias distorts measures of human diversity and may change conclusions drawn from these measures in unexpected ways. They also show that details of the genotyping calling algorithms may have a surprisingly large effect on population genetic inferences. This type of ascertainment bias will be of importance in forthcoming genetic and genomic studies.

However, this paper is concerned with a different type of ascertainment bias which occurs in inter-species or inter-population studies. If a genetic measure of variability or diversity such as heterozygosity, and its underlying causes such as mutation, are studied in more than one species, a careful consideration of the sampling scheme used as basis for comparison is needed. Depending on from which species the polymorphisms are ascertained, the comparison of variability between the two species may be biased in a given direction. We will consider a specific scenario, in which two extant species, such as Human and Chimpanzee, are traced to a common ancestral species. We will consider microsatellite loci, which can be modeled mathematically in a relatively simple way, so that the forward-time simulations can be compared to analytical computations.

We study ascertainment bias of inter-species (population) studies of microsatellite loci, which occurs when a locus is selected based on its large allele size in the species in which it is first discovered (say, the cognate species 1). This bias is reflected in average allele length in any non-cognate species 2 being smaller than that in species 1. This phenomenon was observed in various pairs of species, including Human and Chimpanzee. Various mechanisms were proposed to explain the observed differences in
mean allele lengths between two species. Here, we examine the simplest possible framework: A single-step asymmetric and unrestricted stepwise mutation model with genetic drift. The mathematical model analyzed is based on coalescent theory. The mechanism of ascertainment bias in this model is a tighter correlation of allele sizes within a cognate species 1 than of allele sizes in two different species 1 and 2.

We present computations of the expected bias, given the mutation rate, population sizes of species 1 and 2, time of separation of species 1 and 2, and the age of the allele.

Microsatellite polymorphisms, characterized by variations of copy numbers of short motifs of nucleotides, have become a common tool for gene mapping and evolutionary studies since they are abundantly found in genomes of a large number of organisms (Pena et al. 1993; Bowcock et al. 1994; Deka et al. 1994; Primmer and Ellegren 1998). High mutation rate at these loci is the attractive feature of using the microsatellites as tools for molecular evolutionary studies, since consequences of accumulation of past mutation events are seen as differences of allele frequency distributions even in closely related taxa (Weber and Wong 1993; Kimmel and Chakraborty 1996; Chakraborty et al. 1997). However, in cross-species comparisons of allele size distributions at microsatellite loci some apparently discordant findings (namely, a systematic bias of average allele sizes in one species as compared to another), led some investigators to argue that these repeat loci may not be the most efficient tools for inter-species studies (Rubinsztein et al. 1995; Crawford et al. 1998). In general, for evolutionary studies microsatellite loci as identified in one species (or population) are studied in other species (or populations), making use of their genome homology. Nevertheless, the process of detection (in the cognate species) and its use in a non-cognate species may inherently impact the allele size distribution and associated other summary measures of genetic variation (such as heterozygosity, allele size variance, or number of segregating alleles). This discordance, called the ascertainment bias, is claimed to have been observed in sheep (Forbes et al. 1995), swallows, cetaceans, ruminants, turtles and birds (Ellegren et al. 1995). However, Rubinsztein et al. (1995) and Amos and Rubinsztein (1996) explained such observations as inter-taxa differences of rates and patterns of mutations at microsatellite loci.

The goal of the present study is to address this issue. Our approach is different from other attempts to study similar problems (see e.g. Rogers and Jorde 1996). We consider a general model of mutations (called the Generalized Stepwise Mutation Model, GSMM) that is shown to be applicable to microsatellites (Kimmel et al. 1996; Kimmel and Chakraborty 1996), on which we superimpose the effects of demographic differences of cognate and non-cognate taxa, as both of these factors are known to jointly affect the features of polymorphisms at microsatellite loci in extant taxa (Kimmel et al. 1998).
Fig. 1: Evolutionary history of a locus in two species

Demographic scenario employed in the mathematical model and SimuPOP simulations. Notation: \( N_0, \) \( N_1 \) and \( N_2 \), effective sizes of the ancestral, cognate and non-cognate populations, respectively; \( X_0, X_1 \) and \( X_2 \), increments of allele sizes due to mutations in the ancestral allele, in chromosome 1 and in chromosome 2, respectively.

In particular, using coalescent theory, we show that when the past demographic histories of the cognate and non-cognate taxa are different, the rate and directionality of mutations impact the allele sizes in the two taxa differently than the simple effect of ascertainment bias.

Materials and Methods

Evolution of a DNA-repeat locus

We consider a DNA-repeat locus that has originated \( t \) units of time ago (at backward or reverse time \( t \)), and observed at present (time 0). Adjective "backward" will be usually omitted. Chromosomes containing the locus belong to one of the two populations (labeled 1 and 2), which diverged \( t_0 \) time units before present (time \( t_0 \)) from an ancestral population (labeled 0). The essentials are depicted in Figure 1.

The ancestral population consists of \( 2N_0 \) chromosomes, and populations 1 and 2 of \( 2N_1 \) and \( 2N_2 \) chromosomes respectively. We assume the time-continuous Fisher-Wright-Moran model (Kimmel et al. 1998). At the locus considered, alleles mutate according to the unrestricted Generalized Stepwise Mutation Model (GSMM, Kimmel and Chakraborty 1996). Specifically, the action of genetic drift and mutation can be represented by the following coalescence/mutation model:
1. Chromosomes 1 and 2, sampled at time 0 from populations 1 and 2, respectively, have a common ancestor $T$ units of time before present (Figure 1). Random variable $T$ has exponential distribution with parameter $1/(2N_0)$, shifted by $t_0$, i.e.

$$\Pr[T > \tau] = \begin{cases} 1; & \tau \leq t_0, \\
\exp[-(\tau - t_0)/(2N_0)]; & \tau > t_0. \end{cases}$$  

(1)

In other words, as long as the two chromosomes or their direct ancestors belong to different populations (i.e. for $\tau \leq t_0$, in backward time), they cannot coalesce. From the moment the populations converge (i.e. for $\tau > t_0$ in reverse time), the distribution of the time to coalescence is exponential with parameter $1/(2N_0)$.

2. Chromosomes 1 and 1', sampled at time 0 from population 1, have a common ancestor $T$ units of time before present, either in population 1, if $T \leq t_0$ or in the ancestral population 0, if $T > t_0$. Therefore, the random variable $T$ has a more complex distribution of the form,

$$\Pr[T > \tau] = \begin{cases} \exp[-\tau/(2N_1)]; & \tau \leq t_0, \\
\exp[-t_0/(2N_1) - (\tau - t_0)/(2N_0)]; & \tau > t_0. \end{cases}$$  

(2)

In other words, as long as the two chromosomes or their direct ancestors belong to population 1 (i.e. for $\tau \leq t_0$, in backward time), they coalesce with intensity $1/(2N_1)$. From the moment the species converge (i.e. for $\tau > t_0$ in backward time), the coalescence intensity is $1/(2N_0)$.

3. Initial size (number of repeats) at the locus at time ($t$) of the origin of the locus is equal to a constant. Choosing this constant equal to 0 is not a restrictive assumption. In our model, we assume that before time $t$ there were no mutation events.

4. Mutation epochs along the lines of descent occur according to a Poisson process with constant intensities $\nu_0$, $\nu_1$ and $\nu_2$ in populations 0, 1 and 2, respectively. Each mutation event alters the allele size $S$ by adding to it a random number of repeats $U$, i.e.

$$S \to S + U.$$
\( U \) is an integer-valued random variable (rv) with probability generating function (pgf)

\[
\varphi_k(s) = E(s^U) = \sum_{i=-\infty}^{\infty} \Pr[U = i]s^i.
\]

The pgf \( \varphi_k(s) \) and, equivalently, the distribution of \( U \) is generally different in each population \( k \) \((k = 0, 1, 2)\). Consequently, the change of the allele size, during a time interval of length \( \Delta t \) spent in population \( k \) is a compound Poisson random variable with pgf \( \exp\{\nu\Delta t[\varphi_k(s) - 1]\} \). For the asymmetric single-step Stepwise Mutation Model (SSMM), we have

\[
\varphi_k(s) = b_k s + d_k / s, \tag{3}
\]

where \( b_k = \Pr[U = 1] \) and \( d_k = \Pr[U = -1] = 1 - b_k \) are the respective probabilities of expansion and contraction of the allele in a single mutation epoch.

Remark. The model is formulated as if the length of generation in species 0, 1, and 2 were identical. However, the mutation rates and populations sizes can be rescaled, to accommodate different generation time as explained in the section concerning modeling (see further on). Indeed all results in the following section are invariant under rescaling. We will return to this issue in the discussion.

**Conditional distributions and ascertainment bias of allele sizes**

The main purpose of this section is to use the coalescent theory (as reviewed by Tavaré 1984) to derive conditional expected allele size at a chromosome, given the allele size on another chromosome sampled either from a different or from the same population as the original chromosome. This information is crucial for obtaining estimates of the ascertainment bias in conjunction with other effects.

**Chromosomes sampled from populations 1 and 2.** We use notation as in Figure 1: \( X_0, X_1 \) and \( X_2 \) denote the incremental changes of allele sizes (or, simply, allele sizes) in the ancestral chromosome, 0, and in chromosomes 1 and 2, respectively. Conditionally on \( T, X_0, X_1 \) and \( X_2 \) are independent random variables. Let us note that while chromosome 0 always lives in population 0, chromosomes 1 and 2 begin their lives in population 0 and then continue in populations 1 and 2. Let \( Y_1 = X_0 + X_1 \) and \( Y_2 = X_0 + X_2 \) denote the allele sizes at time 0 (present time) at chromosomes 1 and 2 respectively. We first compute the expected allele size at chromosome 2, jointly with the allele size at chromosome
1 being equal to \(i\) (conditional on \(\{T = \tau\}\)),

\[
E[Y_2; Y_1 = i|T = \tau] = \sum_j E[X_0 + X_2; X_0 = j; X_1 = i - j|T = \tau] = E[X_2|T = \tau] \Pr[Y_1 = i|T = \tau] + \sum_j j \Pr[X_0 = j|T = \tau] \Pr[X_1 = i - j|T = \tau].
\]  

(4)

In the terms of probability generating functions, we obtain

\[
\sum_i E[Y_2; Y_1 = i|T = \tau]s^i = E[X_2|T = \tau]f_{X_0|T=\tau}(s)f_{X_1|T=\tau}(s) + sf_{X_0|T=\tau}(s)f_{X_1|T=\tau}(s).
\]  

(5)

For more details, see Supporting Information 'Derivation of equations (5) and (6)'.

Chromosomes sampled from population 1. Using the same reasoning, we obtain

\[
\sum_i E[Y_1'; Y_1 = i|T = \tau]s^i = E[X_1'|T = \tau]f_{X_0|T=\tau}(s)f_{X_1|T=\tau}(s) + sf_{X_0|T=\tau}(s)f_{X_1|T=\tau}(s).
\]  

(6)

Probability generating functions and expectations of incremental changes of allele sizes. Random variables \(X_0, X_1\) and \(X_2\) result from compounding the Poisson process (Kingman 1993) of mutations, with varying intensities \(\nu_0, \nu_1\) and \(\nu_2\), by distributions of allele size changes with pgf’s \(\varphi_0(s), \varphi_1(s)\) and \(\varphi_2(s)\) respectively. Without getting into detail, we obtain:

\[
f_{X_0|T=\tau}(s) = \begin{cases} 
\exp \{(t - t_0)\nu_0[\varphi_0(s) - 1] + (t_0 - \tau)\nu_1[\varphi_1(s) - 1]\}; & \tau \leq t_0, \\
\exp \{(t - \tau)\nu_0[\varphi_0(s) - 1]\}; & t_0 < \tau \leq t, \\
1; & \tau > t,
\end{cases}
\]  

(7)

\[
f_{X_i|T=\tau}(s) = \begin{cases} 
\exp \{\tau \nu_i[\varphi_i(s) - 1]\}; & \tau \leq t_0, \\
\exp \{(t - t_0)\nu_0[\varphi_0(s) - 1] + t_0 \nu_i[\varphi_i(s) - 1]\}; & t_0 < \tau \leq t, \\
\exp \{(t - t_0)\nu_0[\varphi_0(s) - 1] + t_0 \nu_i[\varphi_i(s) - 1]\}; & \tau > t,
\end{cases}
\]  

(8)

for \(i = 1, 2\). Also, \(f_{X_1'|T=\tau}(s) \equiv f_{X_1|T=\tau}(s)\). The conditional expected values are obtained by differentiation of respective pgf’s and setting \(s = 1\).

Computational expressions for \(E[Y_2; Y_1 = i]\) and \(E[Y_1'; Y_1 = i]\). In the single-step stepwise mutation
model (SSMM), the pgf’s \( \varphi_0(s) \), \( \varphi_1(s) \) and \( \varphi_2(s) \) have the form as in Equ. (3). We note the expansion

\[
e^{\nu t[bs+ds-1]} = \sum_{i \in \mathbb{Z}} \beta_i s^i = \sum_{i \in \mathbb{Z}} e^{-\nu t} I_i(2
u t \sqrt{bd}) \left( \frac{b}{d} \right)^{i/2} s^i,
\]

valid for \( |s| = 1 \), where \( I_i = I_{-i} \) is the modified Bessel function of the first type, of integer order \( i \) (Refs. Abramowitz and Stegun 1972). Using this expansion, it is possible to represent the right-hand sides of Eqs. (5) and (6) as power series in variable \( s \). Finally,

\[
E[Y_2; Y_1 = i] = \int_0^\infty E[Y_2; Y_1 = i | T = \tau] f_T(\tau) d\tau,
\]

\[
E[Y'_2; Y_1 = i] = \int_0^\infty E[Y'_2; Y_1 = i | T = \tau] f_T(\tau) d\tau,
\]

where \( f_T(\tau) \) is the distribution density of the time to coalescence, based on relationships (1) and (2) respectively. A computational expression for \( \Pr[Y_1 = i] \) can be similarly obtained from

\[
\Pr[Y_1 = i] = \int_0^\infty \Pr[Y_1 = i | T = \tau] f_T(\tau) d\tau.
\]

Suppose that a DNA-repeat locus discovered in a genome search of population 1 is retained for further study if it has a minimum number of \( x \) repeats of the motif, i.e. if

\[ Y_1 \geq x. \]

Number of repeats (allele size) serves here as a substitute measure of the locus’ variability. The reason is that, irrespective of directionality of mutational changes, in the GSMM, the extremes of repeat count are strongly positively correlated with variance of repeat count and heterozygosity at the locus. This latter is a consequence of the random-walk mechanism of mutations in this model (Kimmel and Chakraborty 1996).

If the locus is retained and a sample of \( n \) individuals from the non-cognate population 2 is typed for this locus, then the expected value of the mean repeat count in the sample is equal to

\[
E \left( \frac{1}{n} \sum_{i=1}^{n} Y_{2i} | Y_1 \geq x \right) = E[Y_2 | Y_1 \geq x] = \frac{\sum_{i \geq x} E[Y_2; Y_1 = i]}{\sum_{i \geq x} \Pr[Y_1 = i]}.
\]

If a sample of \( n \) individuals of the cognate population 1 is typed for this locus, then the expected values
of the mean repeat count in the sample is equal to

\[ E \left( \frac{1}{n} \sum_{i=1}^{n} Y'_i | Y_1 \geq x \right) = E[Y'_1 | Y_1 \geq x] = \frac{\sum_{i \geq x} E[Y'_1; Y_1 = i]}{\sum_{i \geq x} \Pr[Y_1 = i]} . \]  

(14)

The mean allele size difference, \( D \), which is due to a combined effect of ascertainment bias and intrinsic genetic factors, can be defined as

\[ D = E[Y'_1 | Y_1 \geq x] - E[Y_2 | Y_1 \geq x] . \]  

(15)

**Simulation method**

Despite the complexity of the theory involved in the study of ascertainment bias, simulation of such a process is straightforward using simuPOP, a general-purpose individual-based forward-time population genetics simulation environment (Peng and Kimmel 2005). We consider a microsatellite locus founder population with \( N_0 \) individuals (\( 2N_0 \) chromosomes). We consider a diploid with initial allele size on each chromosome to be 100. The founder population is evolved for \( t - t_0 \) generations before two copies of this population of sizes \( N_1 \) and \( N_2 \) are created, which are evolved for another \( t_0 \) generations.

Direct execution of simulations for tens of thousands of generations is time-consuming. The probability that a random allele exceeds a specified threshold may be low, therefore many attempts may be needed to obtain an estimate of ascertainment bias.

This problem can be addressed through the use of a scaling technique (Hoggart et al. 2007). Compared to a regular simulation that evolves a population of size \( N \) for \( t \) generations, a scaled simulation with a scaling factor \( \lambda \) evolves a smaller population of size \( N/\lambda \) for \( t/\lambda \) generations with magnified (multiplied by \( \lambda \)) mutation, recombination, and selection forces. This method can be justified by a diffusion approximation to the standard Wright-Fisher process (Ewens 2004; Hoggart et al. 2007); however, because the diffusion approximation only applies to weak genetic forces in the evolution of haploid sequences, it cannot be involved when nonadditive diploid or strong genetic forces are used.

Simulation study has been performed with a scaling factor \( \lambda \), where populations with sizes \( N_i/\lambda \) are evolved for \( t_i/\lambda \) generations, under mutation models with mutation rates \( \lambda \nu \), where \( N_i \sim 10^4 - 10^6 \), \( t_i \sim 10^3 - 10^5 \) and \( \nu_i \sim 10^{-4} \) are values typical of human and primate effective population sizes,
evolutionary history and microsatellite mutation rates. Running the simulations with different scaling factors yields identical results if $\lambda \leq 100$ ($\lambda = 1000, 500, 100, 50, 10$ have been tested).

Results

Summary of modeling results

The purpose of modeling is to determine in what circumstances the presence or absence of differences, observed in sizes of alleles at loci discovered in a cognate species (population 1) and then typed in a non-cognate species (population 2), can be attributed to ascertainment bias, or alternatively to differential effects of genetic drift or mutation rate and pattern. Let us first review the intuitions concerning these effects. These intuitions are valid independently of a particular model of mutations:

1. The observed difference between allele sizes, Equ. (15), results from a stronger correlation between allele states of chromosomes in cognate population 1 as compared to non-cognate population 2.

2. Reduced genetic drift in population 1 may reduce the effects of ascertainment bias. Indeed, if the cognate population 1 is much larger than the non-cognate population 2, then the coalescence process within population 1 has the star-like structure characterized by reduced dependence of allele states (Tajima 1989). Therefore, the difference between correlations of allele states of chromosomes in cognate population 1 and non-cognate population 2, will be reduced. Note that the size of the non-cognate population 2 will not influence the difference of expected allele sizes, but it may influence other indices of polymorphism.

3. Mutation rate and pattern, different in populations 1 and 2, influence the differences in allele sizes between different populations.

Figure 2 depicts a series of modeling studies of $D$, the combined effect of ascertainment bias, genetic drift and differential mutation rate on the mean repeat count, based on SimuPOP model, compared to those obtained using Equ. (15). The error bar refers to mean $\pm 2 \times \text{sem}$ (standard error of the mean) of simulated $D$ values from 1,000 replicates. Parameter values approximate the evolutionary dynamics of
Fig. 2: Observed difference $D$ in allele sizes may be positive or negative.

Comparison of SimuPOP simulations with computations based on Eqn. (15). (A) Values of $D$ for the basic parameter values $b_0 = b_1 = b_2 = b = 0.55$, $\nu_0 = \nu_1 = \nu = 0.0001$, $t_0 = 2 \times 10^5$ generations and $t = 5 \times 10^5$ generations, with the effective sizes of all populations concurrently varying from $2 \times 10^4$ to $4 \times 10^5$ individuals and with mutation rates $\nu_2$ varying from $\nu$ to $5\nu$. (B) Values of $D$ for the basic parameter values $b_0 = b_1 = b_2 = b = 0.55$, $\nu_0 = \nu_2 = \nu = 0.0001$, $t_0 = 2 \times 10^5$ generations and $t = 5 \times 10^5$ generations, with the effective sizes of all populations concurrently varying from $2 \times 10^4$ to $4 \times 10^5$ individuals and with mutation rates $\nu_1$ varying from $\nu$ to $5\nu$. (Assuming 20 years per generation)
dinucleotides in humans and chimpanzees: Time from divergence of species $t_0 = 4 \times 10^6$ years $= 2 \times 10^5$ generations for Figure 2 (assuming 20 years per generation), the age of the repeat locus $t = 1 \times 10^7$ years $= 5 \times 10^5$ generations, mutation rate $\nu = 1 \times 10^{-4}$ per generation, and probability of increase of allele size in a single mutation event, $b = 0.55$. Effective size of the current human population, $2N = 4 \times 10^5$ individuals.

Figure 2A depicts the values of $D$ for the basic parameter values $b_0 = b_1 = b_2 = b = 0.55$, and $\nu_0 = \nu_1 = \nu = 0.0001$, with the effective sizes of all populations concurrently varying from $2 \times 10^4$ to $4 \times 10^5$ individuals and with mutation rates $\nu_2$ varying from $\nu$ to $5\nu$. Figure 2B depicts the values of $D$ for the basic parameter values $b_0 = b_1 = b_2 = b = 0.55$, and $\nu_0 = \nu_2 = \nu = 0.0001$, with the effective sizes of all populations concurrently varying from $2 \times 10^4$ to $4 \times 10^5$ individuals and with mutation rates $\nu_1$ varying from $\nu$ to $5\nu$. These two figures make it explicit that the combined effect of ascertainment bias, genetic drift and differential mutation rate on the mean repeat count can result in a range of $D$ values from positive to negative ones. Genetic algorithm and plausible parameter ranges.

For the purpose of obtaining sets of model parameters that yield good fit to the experimental observation of allele length differences, we have applied the genetic algorithm (Mitchell 1996) as a search heuristic to explore an arguably realistic parameter space that specifies a variety of discrete values within a reasonable range to each of the key parameters. We set $t$ to vary in the range from $440,000$ to $740,000$; $t_0$ from $250,000$ to $400,000$; $N_0$ from $10,000$ to $85,000$; $N_1$ from $5,000$ to $12,000$; $N_2$ from $10,000$ to $25,000$; $\nu_0, \nu_1, \nu_2$ from $5 \times 10^{-5}$ to $1 \times 10^{-3}$; $b_0, b_1, b_2$ from $.51$ to $.55$; $x$ from $12$ to $18$. Discussions and Conclusions section involves more detail about settings of these ranges. In the genetic algorithm of optimization (fitting), each parameter range is encoded by a $2$ to $6$-bit vector, yielding $2^2$ to $2^6$ possible values. An initial 'pseudo-population' was created by setting $X$ randomly chosen parameter combinations as $X$ 'individuals'. The value of each modeling parameter in any 'individual' has been converted to binary format to become a 0-1 sequence. Each sequence can be treated as a 'chromosome'. Thus, the 'genome' of an 'individual' consists of a complete heritable parameter setting. By evolving the 'population' under the Wright-Fisher model for $Y$ generations with 'mutation' and 'crossover', it yields by selection the 'individuals' that can best fit the experimental observation. We compare modeling results to observations of Cooper et al. 1998; see the next section for detail.

In the currently implemented ascertainment scheme, we assume $P(L \geq x) \leq 0.25$ to ensure that the probability of choosing polymorphic loci is relatively small (cf. Table 1B). Given a set of input parameter values (including $t, t_0, N, b,$ and $x$), $P(L \geq x)$ can be approximated by the cumulative
distribution function of the Gaussian distribution shown in the ‘Supporting Information’ section on page 31. If a parameter set yields \( P(L \geq x) > 0.25 \) then it will be excluded. The cutoff 0.25 has been chosen heuristically. If a cutoff greater than 0.25 is adopted, the parameter values to fit \( D_{CH} \) and \( D_{HC} \) are easier to find. The opposite hold if the cutoff is less than 0.25. The 0.25 value seems to lead to a parsimonious variant of acceptable parameter values.

**Comparisons of empirical statistics derived from Human and Chimpanzee microsatellite data**

We apply our model to analyze the well-known data set published by Cooper et al. (1998). These authors examined 40 human microsatellite markers and their homologues in a panel of non-human primates, and showed that human loci tend to be longer. Such trend was also confirmed by several other studies. Taken at face value, this data indicated that, since their most recent common ancestor, more microsatellite expansion mutations have occurred in the lineage leading to humans compared with the lineage leading to chimpanzees. Based on this, they suggested that this provided evidence that microsatellites tended to expand with time and were doing so more rapidly in humans. However, an alternative explanation, which attributes the difference to the influence of ascertainment bias, may also result in the observation of allele length difference. Therefore, Cooper et al. (1998) performed the necessary reciprocal experiment showing that human microsatellites tend to be longer than their chimpanzee homologues, regardless of the species from which the loci were cloned.

Dinucleotide (CA) repeat loci discovered and characterized in humans \((n = 22)\), were on average 5.18 repeat units longer than those in chimpanzees, while dinucleotide repeats discovered in chimpanzees \((n = 25)\) were on average 1.23 repeat units longer in humans. Table 1 lists best fits of three independent parameter searching results based on the genetic algorithm, with setup of \(X = 100, Y = 1000\), probability of 'crossover' = 0.6 and 'mutation rate' = 0.02 in each search. Panel A shows best fits from an exploratory parameter search given a broad range of mutation rates (from \(10^{-5}\) to \(10^{-3}\)), while \(b_0, b_1\) and \(b_2\) and \(x\) are set as default values \((b_0 = b_1 = b_2 = .55, x = 12)\). The mutation rates in the top two best fits are below generally accepted ranges, \(\nu_2 = 2 \times 10^{-5} < 5 \times 10^{-5}\). Although the other three fits yield feasible mutation rate estimates, the parameter combinations result in very high probabilities of finding polymorphic loci, \(P(L \geq x) > .25\). In Panel B, \(P(L \geq x) \leq .25\) is assumed to ensure that
Tab. 1: Parameter settings that yield a good fit, for a range of realistic effective population sizes and mutation rates

<p>| | | | | | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>t₀</td>
<td>N₀</td>
<td>N₁</td>
<td>N₂</td>
<td>ν₀</td>
<td>ν₁</td>
<td>ν₂</td>
<td>b₀</td>
<td>b₁</td>
<td>b₂</td>
<td>x</td>
<td>D_HC</td>
<td>D_CH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>540</td>
<td>270</td>
<td>15</td>
<td>6</td>
<td>15</td>
<td>.00012</td>
<td>.00006</td>
<td>.00002</td>
<td>.55</td>
<td>.55</td>
<td>.55</td>
<td>12</td>
<td>5.17</td>
<td>1.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>550</td>
<td>280</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>.00012</td>
<td>.00006</td>
<td>.00002</td>
<td>.55</td>
<td>.55</td>
<td>.55</td>
<td>12</td>
<td>5.06</td>
<td>1.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>570</td>
<td>300</td>
<td>15</td>
<td>3</td>
<td>20</td>
<td>.00030</td>
<td>.00022</td>
<td>.00016</td>
<td>.55</td>
<td>.55</td>
<td>.55</td>
<td>12</td>
<td>5.35</td>
<td>1.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>560</td>
<td>290</td>
<td>20</td>
<td>5</td>
<td>20</td>
<td>.00030</td>
<td>.00016</td>
<td>.00010</td>
<td>.55</td>
<td>.55</td>
<td>.55</td>
<td>12</td>
<td>5.27</td>
<td>1.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>460</td>
<td>250</td>
<td>25</td>
<td>10</td>
<td>12</td>
<td>.00030</td>
<td>.00055</td>
<td>.00045</td>
<td>.55</td>
<td>.55</td>
<td>.55</td>
<td>12</td>
<td>5.42</td>
<td>1.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| | | | | | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>t₀</td>
<td>N₀</td>
<td>N₁</td>
<td>N₂</td>
<td>ν₀</td>
<td>ν₁</td>
<td>ν₂</td>
<td>b₀</td>
<td>b₁</td>
<td>b₂</td>
<td>x</td>
<td>D_HC</td>
<td>D_CH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>580</td>
<td>260</td>
<td>10</td>
<td>7</td>
<td>25</td>
<td>.00075</td>
<td>.00020</td>
<td>.00005</td>
<td>.51</td>
<td>.51</td>
<td>.51</td>
<td>18</td>
<td>5.18</td>
<td>1.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>720</td>
<td>250</td>
<td>15</td>
<td>8</td>
<td>23</td>
<td>.00015</td>
<td>.00010</td>
<td>.00005</td>
<td>.55</td>
<td>.55</td>
<td>.55</td>
<td>18</td>
<td>5.17</td>
<td>1.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>620</td>
<td>250</td>
<td>10</td>
<td>17</td>
<td>20</td>
<td>.00010</td>
<td>.00005</td>
<td>.00005</td>
<td>.55</td>
<td>.55</td>
<td>.55</td>
<td>12</td>
<td>5.30</td>
<td>1.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>660</td>
<td>250</td>
<td>12</td>
<td>25</td>
<td>.00055</td>
<td>.00025</td>
<td>.00010</td>
<td>.51</td>
<td>.51</td>
<td>.51</td>
<td>18</td>
<td>5.45</td>
<td>1.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>740</td>
<td>260</td>
<td>10</td>
<td>12</td>
<td>25</td>
<td>.00035</td>
<td>.00020</td>
<td>.00010</td>
<td>.51</td>
<td>.51</td>
<td>.51</td>
<td>15</td>
<td>5.02</td>
<td>2.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Information of the plausible range of each input parameter was retrieved from literature (details in ‘Discussion and Conclusions’ section). Times t and t₀ are expressed in thousands of generations (assuming 20 years per generation). Population sizes N₀, N₁ and N₂ are expressed in thousands of individuals. D_HC is the calculated average allele length difference on Human loci that are typed in Chimpanzee and D_CH is the reciprocal difference. Panel A shows best fits from an exploratory parameter search given a broad range of mutation rates (from 10⁻⁵ to 10⁻³), with parameters b₀, b₁, b₂ and x set as default values (b₀ = b₁ = b₂ = .55, x = 12). The mutation rates in the two best fits are below the generally accepted range. Although the other three fits yield acceptable mutation rate estimates, the parameter combinations result in very high probabilities of finding polymorphic loci, P(L ≥ x) > .25. In Panel B, P(L ≥ x) ≤ .25 is assumed to ensure that the probability of choosing polymorphic loci is relatively small. Parameters b₀, b₁, b₂ are set equal and range from .51 to .55. x ranges from 12 to 18. The best fits are obtained when ν₂ is equal to the minimum possible value (5 × 10⁻⁵), while fits become slightly worse if ν₂ is increased (10⁻⁴). In Panel C, when P(L ≥ x) ≤ .25 is still required while b₀, b₁ and b₂ are allowed to vary independently, the parameter search tends to favor b₁ > b₂ and small x (< 15) to yield best fits. In both panels B and C with t, t₀, N, b and x assuming ranges of possible values when P(L ≥ x) ≤ .25, ν₀ is always greater than ν₁ and ν₂; ν₁ is greater than or equal to ν₂; ν₁b₁ is always greater than ν₂b₂.
the probability of choosing polymorphic loci is relatively small. \( b_0, b_1, b_2 \) are set to be equal and range from .51 to .55. \( x \) ranges from 12 to 18. The best fits are obtained when \( \nu_2 \) is equal to the minimum possible value (\( 5 \times 10^{-5} \)), while fits become slightly worse if \( \nu_2 \) is increased (\( 10^{-4} \)). In Panel C, when \( P(L \geq x) \leq .25 \) is still required while \( b_0, b_1, b_2 \) are allowed to vary independently, the parameter search tends to favor \( b_1 > b_2 \) and small \( x \) (< 15) to yield best fits.

For a range of evolutionary times, effective population sizes and mutation rates, higher mutation rates and rates of allele length expansions are always observed at Human microsatellite loci compared to those in Chimpanzee (\( \nu_1 \geq \nu_2 \) and \( \nu_1 b_1 > \nu_2 b_2 \)), consistently with Cooper et al. (1998) data.

**Influence of bottlenecks and expansions in human history**

While assuming a constant population size for Chimpanzee, we explore the influence of bottlenecks and expansions in human history on the observed difference in allele lengths (\( D \)). We extend the current modeling scheme and derive the analytical solution to compute \( D \) with Human cognate population size being arbitrarily varied from one generation to another.

Assume that the lineage of humans has been evolved following a multi-step demographic model, where there are \( L \) steps with human population size varied from step to step.

In the backward direction, we denote the present time in generation units as \( t_L = 0 \), the beginning and ending times of the \( m \)th step (\( m = 1, 2, ..., L \)) as \( t_{m-1} \) and \( t_m \); and the population size of the \( m \)th step as \( N_m \). As already defined, \( t \) and \( t_0 \) are the age of the locus and the time when the two species split, respectively, and \( N_0 \) is the ancestral population size.

Chromosomes 1 and 1’ sampled at time 0 from population 1, have a common ancestor \( T \) units of time before present, either in population 1 at stage \( m \), if \( t_m \leq T \leq t_{m-1} \) (for \( m = 1, 2, ..., L \)) or in the ancestral population 0, if \( T \geq t_0 \). Therefore,

\[
P(T > \tau) = \begin{cases} 
  \exp[-\sum_{k=m+1}^{L} \frac{(t_k - t_{k-1})}{2N_k} - \frac{\tau - t_m}{2N_m}] & ; \quad t < \tau \leq t_{m-1}, \\
  \exp[-\sum_{k=1}^{L} \frac{(t_k - t_{k-1})}{2N_k} - \frac{\tau - t_0}{2N_0}] & ; \quad \tau > t_0.
\end{cases}
\]

(16)

for \( m = 1, 2, ..., L \). For derivation of an analogue of Equ. (11) in the extended model see Supporting Information.

Taking a set of model parameters that fit the data from Table 1, \( t = 620,000, t_0 = 250,000, N_0 = N_1 = 10,000, N_2 = 17,000, \nu_0 = .0001, \nu_1 = .0001, \nu_2 = .000005, b_0 = b_1 = b_2 = 0.55, x = 12 \) we obtain
$D(H - C) = 5.30$ and $D(C - H) = 1.44$ in the modeling scheme assuming fixed human population size.

Figure 3 is a schematic representation of major bottlenecks and expansions in the recent human history. The locus was born in the ancestral population, $t$ generations ago. From $t_0$ when the two species split, effective population sizes for Human and Chimpanzee were equal to $N_1$ and $N_2$ (e.g. 5,000 and 20,000, Burgess and Yang 2008), respectively. At $t_1$ ($\sim 200,000$ yrs ago) when humans evolved to migrate out of Africa, there occurred a bottleneck event caused by fact that a subpopulation of migrants was sampled from a larger African population. Our stratified demographic model assumes that the decreased population size due to that bottleneck was constant until the end of the latest glaciation, $t_2$ ($\sim 12,000$ yrs ago). More precisely, it grew until the beginning of the last glaciation ($\sim 50,000$ yrs ago, Bond and Lotti 1995) and then dropped, but the influence of this detail is minor. After that, human population underwent a series of expansions, with its effective size being $\sim 10^5$ from the end of last glaciation ($t_2$) to A.D. 0 ($t_3 \sim 2,000$ yrs ago), $\sim 10^6$ from year 0 CE ($t_3$) to the emergence of industrialization ($t_4 \sim 180$ yrs ago) and $\sim 10^8$ from $t_4$ to present time (current generation). Adapting the human demography with varying population sizes, as described above, in the extended model, we have calculated $D(H - C) = 5.42$ and $D(C - H) = 1.44$, compared to 5.30 and 1.44 obtained from the original model with fixed human population size. Using another set of model parameters from Table 1, $t = 720,000, t_0 = 260,000, N_0 = 10,000, N_1 = 11,000, N_2 = 13,000, \nu_0 = .00025, \nu_1 = .0001, \nu_2 = .0001, b_0 = b_2 = 0.51, b_1 = 0.55, x = 13$ results in $D(H - C) = 5.17$ and $D(C - H) = 1.20$ obtained from the extended model, compared with 5.08 and 1.20 obtained from the original model. $D(C - H)$ remains the same in the extended model because only the human effective population ($N_1$) has been varied. $D(C - H)$ does not depend on $N_1$ but on $N_2$, which is assumed to be constant in both basic and extended models.

We conclude that for the range of parameters we considered, population bottlenecks and expansions in the recent human history have little impact on the modeled difference of allele sizes based on the settings of model parameters used in Table 1 to fit the data. Finally, the mutation rate estimate in the ancestral population is consistently greater than that in Chimpanzee and in Human it is higher than or equal to that in Chimpanzee.
Fig. 3: Schematic representation of human demographic history with recent bottlenecks and expansions

Black line depicts human population, red line depicts ancestral and chimpanzee populations. $t$: age of the locus ($\sim 560,000$ generations $\sim 11.2$ Myrs ago), $t_0$: species split ($\sim 290,000$ generations $\sim 5.8$ Myrs ago), $t_1$: human migration out of Africa ($\sim 10,000$ generations $\sim 200,000$ yrs ago), $t_2$: end of the last glaciation ($\sim 600$ generations $\sim 12,000$ yrs ago), $t_3$: 0 A.D. ($\sim 100$ generations $\sim 2,000$ yrs ago), $t_4$: beginning of industrialization ($\sim 9$ generations $\sim 180$ yrs ago).

Discussion and Conclusions

Computations presented in this paper demonstrate that the scaled forward simulations using SimuPOP closely match the analytical solution of the evolutionary model used. We note that mathematical derivation of equation (15) depends on simplicity of the assumed microsatellite discovery criterion $Y_1 \geq x$. If this criterion is replaced by a condition on heterozygosity or variance, the theoretical derivations become very difficult. On the other hand, it is easy to use any other microsatellite discovery criterion in SimuPOP simulations.

Data of Cooper et al. (1998) indicate that when the human-derived dinucleotide repeat loci are typed in Chimpanzee, they show a trend towards smaller mean allele sizes in the Chimpanzee as compared to that in human populations. These and other data also suggest that the same holds for other measures of within-population variation (i.e., the chimpanzees showing lower heterozygosity and allele size variance, compared to humans, Vowles and Amos 2006). The theoretical model shows that these observations are in agreement with the presence of ascertainment bias, caused by a selective choice of human loci. In the reciprocal experiment, the chimpanzee-derived dinucleotides, typed in human populations, also show a trend towards smaller mean allele sizes in the chimpanzee as compared to that in human populations.

We adapted a genetic algorithm (Mitchell 1996) to perform an extensive parameter space search by specifying a number of values of each of the key modeling parameters ($t, N, v, b$ and $x$, see Table 1 for details) which are variable within plausible ranges. Patterson et al. (2006) reviewed the estimated times
of divergence of the two species \((t_0)\) and determined that divergence occurred approximately between 250,000 and 350,000 generations ago. This corresponds to approximately \(\sim 5\) to 7 million years by assuming 20 years per generation. For the purpose of modeling, the time when a particular locus was born \((t)\) is computed to be varying around 450,000 to 750,000 generations to ensure the threshold of allele size being large enough so that the polymorphic locus occurs only relatively rarely (\(\leq 25\%\) of loci, see Appendix 'Derivation of \(t\)' for details). Using both likelihood and Bayesian methods, Yang (2002), estimated that the ancestral \((N_0)\) and Chimpanzee \((N_2)\) effective population sizes were ranging from 10,000 to 20,000 individuals, and the Human effective population size was ranging from 3,000 to 12,000 individuals (Burgess and Yang 2008). Chen and Li (2001) suggested a much larger effective population size, 50,000 \(\sim\) 90,000, of the common ancestor of Human and Chimpanzee. We assign multiple numbers within these ranges as possible values of \(N_0, N_1\) and \(N_2\). Additionally, given that microsatellite loci mutation rate in any population is greater than \(10^{-4}\), as analyzed by Ellegren (2000), \(\nu_0, \nu_1, \nu_2\) are assumed in a wide range starting from \(5 \times 10^{-5}\). Mutational biases (Sainudiin et al. 2004; Wu and Drummond 2011) \(b_0, b_1, b_2\), are ranging from \(0.51\) to \(0.55\). In this model, we assume such bias to be constant within a population. As demonstrated in Table 1, for a range of effective population sizes and evolutionary times, the estimated Human mutation rates are always higher than or equal to those in Chimpanzee and the mutation rate estimates in the ancestral population are always greater than those in either Human or Chimpanzee.

These observations imply that ascertainment bias is a significant factor in interpreting inter-population genetic variation at microsatellite loci, when the loci are selectively chosen for polymorphism in one of the populations compared. Ascertainment bias effect is confounded by other differences in evolutionary dynamics between the cognate and non-cognate populations, particularly by inter-population differences of rates of mutations at the locus. As shown in Figure 2A, increased mutation rate in the non-cognate population reduces the effect of the ascertainment bias, while increased mutation rate in the cognate population amplifies the effect of the bias (Figure 2B). On the other hand, the primary cause of ascertainment bias is a tighter correlation of allele sizes within the cognate population. Thus, intuitively it is clear that population size differences between cognate and non-cognate populations may reduce or amplify the ascertainment bias. If the cognate population is of larger size or is growing more rapidly than the non-cognate one, a reduced bias is expected.

The differences of patterns of biases seen at the dinucleotide loci discovered in Human versus Chimpanzee can be explained by our model if the mutation rate is higher for humans. The observed pattern
that ascertainment bias is of a lower magnitude for the chimpanzee-specific loci is also consistent with effective population size in Chimpanzee being smaller than that in Human. In this sense, our observations and theoretical predictions are consistent with the assertion of Rubinsztein et al. (1995), although expansion bias of mutations is not necessary to explain the observed differences in humans and chimpanzees.

As mentioned, when describing the model, the time and mutation rates (as well as effectively the population sizes) are scaled to the unit equal to the human generation length. This is convenient, and the numbers can be rescaled to accommodate different evolutionary parameters in different species. Our theory and data can also be used to explain the apparently discordant conclusions reached by other investigators examining this issue. For example, Ellegren et al. (1995) observed smaller allele sizes in non-cognate species compared with cognates of birds, which could be predominantly due to ascertainment bias alone. Crawford et al. (1998), in contrast, found longer median allele sizes in sheep compared with cattle, regardless of the origin of the microsatellites. This may be the case where the ascertainment bias effect is counteracted or even reversed due to mutation rate and/or effective population size differences in sheep and cattle.

There had been discussions with regard to the dependence of inter-population allele size differences on the absolute repeat lengths of alleles (Ellegren et al. 1995; Amos and Rubinsztein 1996). For microsatellites, there is a general tendency for increased level of polymorphism at loci harboring larger alleles (Weber 1990). Our theory shows that loci exhibiting higher degrees of polymorphism are likely be subject to lesser bias of ascertainment (due to lower correlation of allele sizes in the cognate population). Hence, appropriate adjustment of inter-locus differences of polymorphism as well as allele sizes should be made in addressing the importance of ascertainment bias.

Wolkes and Amos (2006) is an important contribution to the literature on ascertainment bias. Among other, these authors observed that long repeats tend to be interrupted, which contributes an additional bias. They also proposed that the difference $D$ be explained if microsatellites evolve at different rates, with longer microsatellites evolving faster; this latter effect having some statistical rationale. In the current paper, we offer an explanation which does not rely on interruption nor acceleration, but only on sampling, and demographic and population-genetic effects, under constant though species-dependent mutation rates. However, there is at least some concordance; we find that human microsatellites, which are on the average longer, also have higher mutation rates, which might be a hint that both approaches detect the same or similar effect.
In summary, we conclude that ascertainment bias is an important consideration for interpretation of inter-population differences of genetic variation at microsatellite loci, but this bias can be reduced or even reversed when the past demographic histories of cognate and non-cognate populations are different. In addition, mutation rate differences among populations can also influence or mimic ascertainment bias.

Acknowledgments

Research supported by NIH grants GM 58545, GM 45861 and GM 41399, and Polish National Center for Science grant NN519579938.

No conflicts of interest declared by the authors.

References


**Supporting Information**

**Derivation of equation (4)**

Since $Y_2 = X_0 + X_2$ and $Y_1 = X_0 + X_1$, we have

$$E[Y_2; Y_1 = i|T = \tau] = \sum_j jP(X_0 = j; X_1 = i - j|T = \tau) P(X_0 + X_1 = i|T = \tau) + E[X_2|T = \tau] P(X_0 + X_1 = i|T = \tau)$$

**Derivation of equations (5) and (6)**

If $X$ is a discrete random variable taking values in the non-negative integers $\{0, 1, 2, \ldots\}$, then we define the probability generating function (pgf) of $X$ as:

$$f_X(s) = E[s^X] = \sum_{x=0}^{\infty} P(X = x) s^x$$

Restating equation (4) in the terms of the probability generating functions, and using independence
of \((X_0|T), (X_1|T), (X_2|T)\), we obtain

\[
\sum_i E[Y_2, Y_1] = i|T = \tau] s^i
= \sum_i E[X_2|T = \tau] P(X_0 + X_1 = i|T = \tau] s^i
+ \sum_i \sum_j j P(X_0 = j|T = \tau] P(X_1 = i - j|T = \tau] s^{i-j} s^j
= E[X_2|T = \tau] f_{X_0+X_1|T=\tau}(s) + s \sum_i f'_{X_0|T=\tau}(s) P(X_1 = i|T = \tau] s^{i-j}
= E[X_2|T = \tau] f_{X_0+X_1|T=\tau}(s) + s f'_{X_0|T=\tau}(s) \sum_{i=-\infty}^{\infty} P(X_1 = i|T = \tau] s^{i-j}
= E[X_2|T = \tau] f_{X_0|T=\tau}(s) f_{X_1|T=\tau}(s) + s f'_{X_0|T=\tau}(s) f_{X_1|T=\tau}(s)
\]

Thus, equation (5) holds; derivation of equation (6) is similar.

**Derivation of equations (7) and (8)**

Consider the Poisson Process \(N\) with intensity \(\nu\), where \(N(T) = \# \{\text{events of mutations occurring in time interval of length } T\}\),

\[f_{N(T)}(s) = e^{\nu t(s-1)}\] and \(T\) is the coalescence time before present. Let \(X_0, X_1, X_2\) denote the incremental changes of allele length of chromosomes 0, 1 and 2. \((X_0|T), (X_1|T), (X_2|T)\) are conditionally independent and \(f_{U_k}(s) = \psi_k(s), k = 0, 1, 2\).

Therefore, if \(\tau \leq t_0\), it holds

\[X_0|(T = \tau) = X_{0,1}|(T = \tau) + X_{0,2}|(T = \tau), \text{ where } X_{0,1}|T = \tau \text{ is the increment of allele size in the interval } [t, t_0) \text{ and } X_{0,2}|T = \tau \text{ is the increment in the interval } [t_0, \tau). \text{ Since } X_{0,1}|(T = \tau) \text{ is independent of } X_{0,2}|(T = \tau), \text{ it holds that pgf } f_{X_0|T=\tau}(s) = f_{X_{0,1}|T=\tau}(s) \cdot f_{X_{0,2}|T=\tau}(s), \text{ where } f_{X_{0,1}|T=\tau}(s) = f_{N(t-t_0)}(\psi_0(s)) = e^{\nu(t-t_0)(\psi_0(s)-1)}, \text{ and } f_{X_{0,2}|T=\tau}(s) = f_{N(t-t_0)}(\psi_1(s)) = e^{\nu(t-t_0)(\psi_1(s)-1)}.

It follows that \(f_{X_0|T=\tau}(s) = e^{\nu(t-t_0)(\psi_0(s)-1) + \nu_2(t_0 - \tau)(\psi_1(s) - 1)}\), and \(f_{X_1|T=\tau}(s) = f_{N}(\psi_1(s)) = e^{\nu\tau(\psi_1(s) - 1)}\). Therefore, both equations (7) and (8) hold for \(\tau \leq t_0\). Derivations for \(t_0 < \tau \leq t\) or \(\tau > t\) are similar.
Derivation of computational expressions for equations (10) and (11)

Expected size of allele drawn from population 2, jointly with size of allele drawn from population 1 being equal to \( i \) is equal to \( E[Y_2, Y_1 = i] = \int_0^\infty E[y_2, Y_1 = i | T = \tau] f_T(\tau) d\tau \), where \( f_T(\tau) \) is given in equation (1). However, \( T \) denotes the common ancestor time of chromosomes 1 and 2 sampled at time 0 from populations 1 and 2. Therefore, from Equ. (1), we have

\[
f_T(\tau) = \begin{cases} 
0 & \tau \leq t_0 \\
\frac{1}{2N_0} e^{-(\tau-t_0)/(2N_0)} & \tau > t_0
\end{cases}
\]

If \( \tau \leq t_0 \), \( f_T(\tau) = 0 \) and \( E[Y_2; Y_1 = i] = 0 \).

If \( t_0 < \tau \leq t \), from Equ. (5) we obtain

\[
\sum_i E[Y_2, Y_1 = i | T = \tau] s^i = E[X_2 | T = \tau] f_{X_0 | T = \tau}(s) f_{X_1 | T = \tau}(s) + s f_{X_0 | T = \tau}(s) f_{X_1 | T = \tau}(s)
\]

where,

\[
E[X_2 | T = \tau] f_{X_0 | T = \tau}(s) f_{X_1 | T = \tau}(s) = E[X_2 | T = \tau] e^{(\nu_0(t-t_0)b_0 + \nu_1 t_0 b_1)s} e^{-(\nu_0(t-t_0) + \nu_1 t_0) s}.
\]

By differentiating \( f_{X_2 | T = \tau}(s) \) and setting \( s = 1 \), we obtain

\[
E[X_2 | T = \tau] = (\nu_0(t-t_0)(b_0 - d_0) + \nu_1 t_0 b_1 e^{(\nu_0(t-t_0)b_0 + \nu_1 t_0 b_1)s} e^{-(\nu_0(t-t_0) + \nu_1 t_0) s}) = e^{(bs)/d}.
\]

According to Equ. (9),

\[
e^{(bs)/d} = \sum_{i \in \mathbb{Z}} I_i(2\sqrt{bd})(\frac{b}{d})^{i/2} s^i = \sum_{i \in \mathbb{Z}} \beta_i s^i, \quad |s| = 1,
\]

where we denote \( \beta_i = I_i(2\sqrt{bd})(\frac{b}{d})^{i/2} \), and \( I_i = I_{-i} \) is the modified Bessel function of the first type.
(Abramowitz and Stegun 1972), of integer order $i$.

Thus

$$E(X_2|T = \tau)f_{X_0|T=\tau}(s) = e^{-\nu_0(t-t_0)-\nu_1t_0} \sum_{i \in Z} E(X_2|T = \tau)\beta_i s^i$$

Furthermore, $sf'_{X_0|T=\tau}(s)f_{X_1|T=\tau}(s)$ in equation (5) can be expressed as

$$sf'_{X_0|T=\tau}(s)f_{X_1|T=\tau}(s) = sv_0(t - \tau)\psi'(s) \times \exp(\nu_0(t-t_0)(\psi_0(s) - 1) + \nu_1t_0(\psi_1(s) - 1))$$

$$= sv_0(t - \tau)(b_0 - d_0/s^2) \times e^{((\nu_0(t-t_0)b_0 + \nu_1t_0b_1) + (\nu_0(t-t_0)d_0 + \nu_1t_0d_1)/s)} e^{-\nu_0(t-t_0)-\nu_1t_0}$$

$$= \nu_0(t - \tau)(b_0s - d_0/s)e^{-\nu_0(t-t_0)-\nu_1t_0}(\sum_{i \in Z} \beta_i s^i)$$

$$= e^{-\nu_0(t-t_0)-\nu_1t_0}(\sum_{i \in Z} \nu_0(t - \tau)b_0\beta_i s^{i+1} - \sum_{i \in Z} \nu_0(t - \tau)d_0\beta_i s^{i+1})$$

$$= e^{-\nu_0(t-t_0)-\nu_1t_0}(\nu_0(t - \tau)b_0 \sum_{i=1}^{i=1} \beta_{i-1}s^i - \nu_0(t - \tau)d_0 \sum_{i=1}^{i+1} \beta_{i+1}s^i)$$

Therefore, when $t_0 < \tau \leq t$

$$\sum_i E[Y_2, Y_1 = i|T = \tau] s^i = E[X_2|T = \tau] f_{X_0|T=\tau}(s) f_{X_1|T=\tau}(s) + sf'_{X_0|T=\tau}(s)f_{X_1|T=\tau}(s)$$

$$= e^{-\nu_0(t-t_0)-\nu_1t_0} \sum_{i \in Z} [E[X_2|T = \tau] \beta_i + \nu_0(t - \tau)b_0\beta_{i-1} - \nu_0(t - \tau)d_0\beta_{i+1}] s^i$$

$$= e^{-\nu_0(t-t_0)-\nu_1t_0} \sum_{i \in Z} (\nu_0(t - \tau)b_0\beta_{i-1} - \nu_0(t - \tau)d_0\beta_{i+1}$$

$$+ [\nu_0(\tau - t_0)(b_0 - d_0) + \nu_2t_0(b_2 - d_2)]e^{i\nu_0(\tau-t_0)(b_0+d_0-1)+\nu_2t_0(b_2+d_2-1)\beta_i} s^i$$

which yields

$$E[Y_2, Y_1 = i|T = \tau] = e^{-\nu_0(t-t_0)-\nu_1t_0}\{\nu_0(t - \tau)b_0\beta_{i-1} - \nu_0(t - \tau)d_0\beta_{i+1}$$

$$+ [\nu_0(\tau - t_0)(b_0 - d_0) + \nu_2t_0(b_2 - d_2)]e^{i\nu_0(\tau-t_0)(b_0+d_0-1)+\nu_2t_0(b_2+d_2-1)\beta_i} \}$$

which provides the desired computable form of Equ. (10)
If \( \tau > t \), analogous computations yield

\[
E[Y_2, Y_1 = i] = \int_t^\infty E[Y_2, Y_1 = i|T = \tau] f_T(\tau) d\tau \\
= E[Y_2, Y_1 = i|T = \tau] P[T > \tau] \\
= e^{-\sum_{i=0}^{\infty} \nu_i t_0 - \nu_1(t-t_0) - \nu_2(t_0)(b_0 - d_0)} \beta_i [\nu_0(t - t_0)b_0 + \nu_1(t_0 - \tau)b_1]d_{i-1} + \nu_1 \tau(b_1 - d_1) e^{-\nu_1 \tau(b_1 + d_1 - 1)} \beta_i - \nu_0(t - t_0)d_0 + \nu_1(t_0 - \tau)d_1] \beta_{i+1}
\]

Similarly as derivation of Equ. (10), Equ. (11) can be fully derived from Equ. (2), (6) and (9) for its computable form, where

if \( \tau \leq t_0 \),

\[
E[Y'_1, Y_1 = i|T = \tau] = e^{-\nu_0(t-t_0)-\nu_i t_0} [\nu_0(t - t_0)b_0 + \nu_1(t_0 - \tau)b_1]d_{i-1} - \nu_0(t - t_0)d_0 + \nu_1(t_0 - \tau)d_1] \beta_{i+1}
\]

if \( t_0 < \tau \leq t \),

\[
E[Y'_1, Y_1 = i|T = \tau] = e^{-\nu_0(t-t_0)-\nu_i t_0} [\nu_0(t - \tau)b_0 + \nu_1(t_0 - \tau)d_0]d_{i+1} + [\nu_0(t - t_0)(b_0 - d_0) + \nu_1t_0(b_1 - d_1)] e^{-\nu_0(t-t_0)(b_0 + d_0 - 1) + \nu_i t_0(b_1 + d_1 - 1)} \beta_i
\]

if \( \tau > t \),

\[
E[Y'_1, Y_1 = i|T = \tau] = e^{-\nu_0(t-t_0)-\nu_i t_0} [\nu_0(t - t_0)(b_0 - d_0) + \nu_1t_0(b_1 - d_1)] e^{-\nu_0(t-t_0)(b_0 + d_0 - 1) + \nu_i t_0(b_1 + d_1 - 1)} \beta_i
\]

**Derivation of computational expression for equation (12)**

The probability generating function of \( Y_1|T = (X_0|T) + (X_1|T) \) is equal to \( f_{X_0|T=\tau}(s)f_{X_1|T=\tau}(s) \) because \( X_0|T \) is conditionally independent of \( X_1|T \).

Therefore,

\[
\sum_i P(Y_1 = i|T = \tau) s^i = f_{X_0|T=\tau}(s)f_{X_1|T=\tau}(s) \\
= e^{\nu_0(t - t_0)(\psi_0(s) - 1) + \nu_1t_0(\psi_1(s) - 1)} \\
= e^{-\nu_0(t-t_0)-\nu_i t_0} \sum_{i \in Z} \beta_i s^i
\]

which yields \( P(Y_1 = i) = \int_0^\infty P(Y_1 = i|T = \tau)f_T(\tau)d\tau = e^{-\nu_0(t-t_0)-\nu_i t_0} \beta_i \), the derived computational expression for Equ. (12).
Derivation of equations (13) and (14)

By definition,

\[ E \left[ Y_2 | Y_1 \geq x \right] = \frac{E[Y_2, Y_1 \geq x]}{P(Y_1 \geq x)} \]

\[ = \frac{\sum_j j P(Y_2 = j, Y_1 \geq x)}{\sum_{i \geq x} P(Y_1 = i)} \]

\[ = \frac{\sum_j \sum_{i > x} P(Y_2 = j, Y_1 = i)}{\sum_{i \geq x} P(Y_1 = i)} \]

\[ = \frac{\sum_{i \geq x} E[Y_2 | Y_1 = i]}{\sum_{i \geq x} P(Y_1 = i)} \]

Equ. (13) has been derived. Similarly, Equ. (14) can be derived.

Derivation of the range for the estimate of \( t \)

We denote random variable \( L \) as the incremental allele length of a sampled individual, and show the computation of the expectation \( E[L] \) and variance \( V[L] \) given \( t, t_0, \nu_0, \nu_1, b_0 \) and \( b_1 \), where \( t \) and \( t_0 \) are expressed in generation units.

Let \( L_0, L_1 \) be two random variables that denote the allele length increments in time interval \( t \) to \( t_0 \) (ancestral population 0) and \( t_0 \) to present (cognate population 1), respectively.

Let \( X_i \) be the incremental change in the the \( i \)th generation of the ancestral population (from \( t \) to \( t_0 \)). We may obtain \( P(X_i = 1) = \nu_0 b_0 \), \( P(X_i = -1) = \nu_0(1 - b_0) \), \( P(X_i = 0) = 1 - \nu_0 \) and

\[ E[X_i] = 2\nu_0 b_0 - \nu_0 \]

\[ V[X_i] = \nu_0 - \nu_0^2 (2b_0 - 1)^2 \]

Based on the fact that the incremental change of allele length in any generation is independent of that in any other generation, we have

\[ E[L_0] = (t - t_0) E[X_i] = (t - t_0)\nu_0(2b_0 - 1) \]

\[ V[L_0] = (t - t_0) V[X_i] = (t - t_0)\nu_0[1 - \nu_0(2b_0 - 1)^2] \]
Similarly we derive \( E[L_1] \) and \( V[L_1] \) as

\[
E[L_1] = t_0\nu_1(2b_1 - 1)
\]
\[
V[L_1] = t_0\nu_1[1 - \nu_1(2b_1 - 1)^2]
\]

Therefore, we obtain

\[
E[L] = E[L_0] + E[L_1] = (t - t_0)\nu_0(2b_0 - 1) + t_0\nu_1(2b_1 - 1)
\]
\[
V[L] = V[L_0] + V[L_1] = (t - t_0)\nu_0[1 - \nu_0(2b_0 - 1)^2] + t_0\nu_1[1 - \nu_1(2b_1 - 1)^2]
\]

Assuming that \( L \) is approximately Gaussian and given \( x \) as the threshold of allele size discovery, we calculate \( P(L \geq x) \) from the cdf of the Gaussian distribution. This helps to determine a realistic range of estimates of \( t \) to control the probability of locus discovery.

**Derivation of \( E[Y'_1; Y_1 = i] \) in the extended model**

From the Equ. 16 derived earlier on, we obtain

\[
f_T(\tau) = \begin{cases} 
\frac{1}{2N_m} \exp\left[-\sum_{k=m+1}^{L} \left( t_{k-1} - t_k \right) \right] - \frac{\tau - t_m}{2N_m}; & t < \tau \leq t_{m-1}, \\
\exp\left[-\sum_{k=1}^{L} \left( t_{k-1} - t_k \right) \right] - \frac{\tau - t_0}{2N_0}; & \tau > t_0.
\end{cases}
\]

If \( t \leq t_0 \) and \( t_{m-1} < \tau \leq t_{m-1} \) \((m = 1, 2, ..., L)\), we obtain

\[
E[Y'_1; Y_1 = i] = i|T = \tau| = e^{-\nu_0(t-\tau)}\nu_1 \left[ \nu_0(t-\tau)d_0 + \nu_1(t_0-\tau)b_1 \right] \beta_i + \nu_1 \left( b_1 - d_i \right) \beta_i - \nu_0(t-\tau)d_0 + \nu_1(t_0-\tau)b_1 \beta_{i+1}
\]

\[
E[Y'_1; Y_1 = i] = \sum_{m=1}^{L} \int_{t_m}^{t_{m-1}} E[Y'_1; Y_1 = i|T = \tau] \left( \frac{1}{2N_m} \right) \exp\left[-\sum_{k=m+1}^{L} \left( t_{k-1} - t_k \right) \right] - \frac{\tau - t_m}{2N_m} d\tau
\]

where \( \beta_i \) is the same as that defined and used in the derivation of Eqs. (10) and (11) in the main text.
If \( t_0 < \tau \leq t \), we obtain

\[
E[Y'_1; Y_1 = i | T = \tau] = e^{-\nu_0 (t - t_0) - \nu t_0} \{\nu_0 (t - \tau)b_0 \beta_{i-1} + \nu_0 (\tau - t_0)(b_0 - d_0) + \nu_1 t_0 (b_1 - d_1)\} \beta_i - \nu_0 (t - \tau)d_0 \beta_{i+1}
\]

\[
E[Y'_1; Y_1 = i] = \int_{t_0}^{t} E[Y'_1; Y_1 = i | T = \tau] \left( \frac{1}{2N_0} \right) \exp \left[ - \sum_{k=1}^{L} \left( \frac{t_{k-1} - t_k}{2N_k} \right) - \frac{\tau - t_0}{2N_0} \right] d\tau
\]

If \( \tau > t \), we obtain

\[
E[Y'_1; Y_1 = i | T = \tau] = e^{-\nu_0 (t - t_0) - \nu t_0} [\nu_0 (t - t_0)(b_0 - d_0) + \nu_1 t_0 (b_1 - d_1)] \beta_i
\]

which is not a function of \( \tau \).

Therefore

\[
E[Y'_1; Y_1 = i] = \int_{t}^{\infty} E[Y'_1; Y_1 = i | T = \tau] f_T(\tau) d\tau = E[Y'_1; Y_1 = i | T = \tau] P(T > t)
\]

\[
= e^{-\sum_{k=1}^{L} \left( \frac{t_{k-1} - t_k}{2N_k} \right) - \frac{t_0}{2N_0} - \nu t_0} [\nu_0 (t - t_0)(b_0 - d_0) + \nu_1 t_0 (b_1 - d_1)] \beta_i
\]

With the analytical derivation shown here we are able to compute the allele length difference \( D \) with Human population size arbitrarily varied from generation to generation.