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Published in:
Bioinformatics

DOI:
10.1093/bioinformatics/btw670

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Sequence analysis

gargammel: a sequence simulator for ancient DNA

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Associate Editor: Inanc Birol

Received on August 19, 2016; revised on October 13, 2016; editorial decision on October 17, 2016; accepted on October 20, 2016

Abstract

Summary: Ancient DNA has emerged as a remarkable tool to infer the history of extinct species and past populations. However, many of its characteristics, such as extensive fragmentation, damage and contamination, can influence downstream analyses. To help investigators measure how these could impact their analyses in silico, we have developed gargammel, a package that simulates ancient DNA fragments given a set of known reference genomes. Our package simulates the entire molecular process from post-mortem DNA fragmentation and DNA damage to experimental sequencing errors, and reproduces most common bias observed in ancient DNA datasets.

Availability and Implementation: The package is publicly available on github: https://grenaud.github.io/gargammel/ and released under the GPL.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

DNA retrieved from subfossils, also called ancient DNA (aDNA), is increasingly used to reconstruct population histories (Leonardi et al., 2016). The analysis of aDNA data remains, however, challenging due to a number of factors that can affect downstream inferences. First, DNA tends to degrade over time, leading to fragments of limited sizes (30–80 bp) showing substantial nucleotide misincorporations (Briggs et al., 2007). Second, environmental microbes tend to colonize the organism postmortem (Green et al., 2009). As a result, the endogenous DNA fraction can sometimes be extremely reduced, making shotgun sequencing approaches un economical. Third, such exogenous sequences can impact the reconstruction of ancient genomes if not properly identified during read alignment. In the case of aDNA retrieved from hominin species, the DNA from present-day humans, which can be introduced at any stage including during the excavation and in the laboratory, is particularly problematic as it mixes unrelated population histories within a single sample.

Ancient DNA researchers often use simulations to test the robustness of summary statistics aimed at inferring population parameters. While some packages have simulated platform-specific errors due to sequencing, no packages are currently available to properly simulate aDNA sequence datasets, including their most prominent characteristics, such as such as damage, fragmentation, human and microbial contamination.

Here, we present gargammel, a package that simulates aDNA sequence datasets from a set of genome references representing the microbial fraction, the endogenous fraction and the present-day human contamination. The package can simulate most common features of aDNA sequences, including post-mortem DNA damage and base misincorporations. In addition, it simulates base compositional bias due to the molecular tools used in library preparation,
sequencing bias against GC-rich fragments and errors introduced by
the sequencing platform.

2 Methods

Our algorithm reflects the entire molecular and experimental pro-
cess leading to the retrieval of aDNA fragments (Fig. 1). In its sim-
plest mode, the user first provides three sets of references in fasta
format: (i) the microbial contaminant, (ii) endogenous genome and
(iii) the present-day human contamination. The user can also pro-
vide full microbial profiles, including taxonomic abundances, to rep-
resent more complex sources of microbial contamination. In this
case, corresponding (or closely related) microbial genomes will be
automatically downloaded from NCBI. The user either provides the
desired endogenous coverage or a fixed number of fragments to
simulate. The endogenous genome can contain 1 sequence for hap-
loid organisms or 2 sequences as to simulate a diploid organism
where fragments are sampled from each with equal probability.

Fragments are selected from all three sets depending on the
desired composition of the final set (e.g. 70% microbial, 20% endo-
genous and 10% present-day human contamination). The size of
the fragments can be selected from a user-specified distribution.

As aDNA base composition can be different from modern DNA
(Jönsson et al., 2013) and vary together with the molecular tools
used during library preparation (Seguin-Orlando et al., 2013), the
base composition can also be modeled. Subsequently, post-mortem
deamination is added according to the parameters of standard
aDNA damage models (Briggs et al., 2007) or a user-specified ma-
trix of position-specific misincorporation rates.

Fragmented aDNA templates can be shorter than the read
length. Gargammel proceeds by adding the necessary length of the
sequencing adapter. Finally, the ART sequencing simulator (Huang
et al., 2012) is used on the resulting sequences to produce Illumina
reads with sequencing errors and quality scores. The various subpro-
grams of the pipeline are called by a wrapper script and are detailed
in the Supplementary Methods. Finally, the wrapper script combines
reads from the three sources to create the final sequence set.

3 Features

We tested gargammel for its ability to reproduce empirical features
found in six previously released aDNA datasets (see Supplementary
Results). These include: (i) size distribution, (ii) base composition,
(iii) GC-bias due to the DNA polymerase used for library amplifica-
tion and; (iv) DNA misincorporation. The results presented in the
Supplementary Results show a high consistency between observed
and simulated distributions to show the applicability of gargammel
as a sequence simulator for aDNA.

Gargammel provides researchers with the opportunity to per-
form various inquiries to evaluate the robustness of various analyses
to aDNA properties. In the Suppl. Results, we present two such
types of analyses. First, we evaluated the potential impact of
present-day contamination on admixture tests based on the D-statis-
tics (Durand et al., 2011). Simulated sequences were obtained from
coalescence models without any admixture. We find that amounts
of present-day human contamination in ancient human datasets can
create spurious signals of admixture depending on the coalescent
model, especially when a distant outgroup is used in the test and
when the modern contamination source originates from a popula-
tion coalescing deeply with the endogenous individual. Second, we
evaluated whether microbial fragments of increasing size could im-
pact the false positive alignment rate against the human reference
genome (see Schubert et al., 2012 for an evaluation of aDNA map-
ning). We identified a size threshold (35 bp) that reduces such im-
pact for the microbial community identified in the 12.8 kyr-old
Clovis individual (Rasmussen et al., 2014). Such tests can be
adapted to any other situation of interest.

Acknowledgments

We thank Weichun Huang and all members of the Paleomix group at the
Centre for GeoGenetics for fruitful discussions.

Funding

This work was supported by the Danish Council for Independent Research,
Natural Sciences (4002-00152B); the Danish National Research Foundation
Conflict of Interest: none declared.

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