Accurate Bayesian inference of sex chromosome karyotypes and sex-linked scaffolds from low-depth sequencing data

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1 Abstract

The identification of sex-linked scaffolds and the genetic sex of individuals, i.e. their sex karyotype, is a fundamental step in population genomic studies. If sex-linked scaffolds are known, single individuals may be sexed based on read counts of next-generation sequencing data. If both sex-linked scaffolds as well as sex karyotypes are unknown, as is often the case for non-model organisms, they have to be jointly inferred. For both cases, current methods rely on arbitrary thresholds, which limits their power for low-depth data. In addition, most current methods are limited to euploid sex karyotypes (XX and XY). Here we develop `BeXY`, a fully Bayesian method to jointly infer the posterior probabilities for each scaffold to be autosomal, X- or Y-linked and for each individual to be any of the sex karyotypes XX, XY, X0, XXX, XXY, XYY and XXYY. If the sex-linked scaffolds are known, it estimates the sex karyotype posterior probabilities also for single individuals. As we show with downsampling experiments, `BeXY` has higher power than all existing methods. It accurately infers the sex karyotype of ancient human samples with as few as 20,000 reads and accurately infers sex-linked scaffolds from data sets of just a handful of samples or with highly imbalanced sex ratios, also in the case of low-quality reference assemblies. We illustrate the power of `BeXY` by applying it to both whole-genome shotgun and target enrichment sequencing data of ancient humans as well as several non-model organisms.

Keywords: molecular sexing, aneuploidy, sex chromosomes, low-depth sequencing, ancient DNA

2 Introduction

Many population genomic analyses rely on an accurate identification of the genetic sex of the individuals as well as an identification of sex-linked scaffolds. The identification of genetic sex is used to reveal patterns of sex-specific ecology and evolution, for example concerning behaviour and social structure

(e.g. Pečnerová et al., 2017; Gower et al., 2019), foraging ecology (e.g. Louis et al., 2021) or sex-biased dispersal and philopatry (e.g. Bidon et al., 2014; Herrero et al., 2021). In the context of ancient humans cultures, the identification of the genetic sex has been used to study social structure (e.g. Žegarac et al., 2021; Hedenstierna-Jonson et al., 2017), religion (e.g. De La Cruz et al., 2008), sex-biased migration (e.g. Veeramah et al., 2018) or to evidence women participating in prehistorical battles (e.g. Burger et al., 2020). The identification of sex-linked scaffolds further allows to study sex-linked disease susceptibility (Schurz et al., 2019), sex-biased gene expression (Grath and Parsch, 2016) or sex-linked gene-flow (Bidon et al., 2014). In addition, sex-linked scaffolds often require special treatment in population genomic analyses, for example for fair filtering on read depth and minor allele frequency, genotype calling and Hardy-Weinberg checks, but also because they bias estimates of genetic diversity such as heterozygosity (Ellegren, 2009), estimates of mutation rates (Ellegren, 2007), genome-wide association studies (Gao et al., 2015) and assessments of population genetic structure (Benestan et al., 2017). If genetic sex and/or sex-linked scaffolds are unknown, they need to be characterized using dedicated methods that can be easily integrated in population genomic analyses.

The mechanisms of sex determination are remarkably variable (The Tree of Sex Consortium et al., 2014), and genetic sex determination through sex chromosomes has evolved independently many times throughout eukaryotes (Graves, 2008). However, two major systems can be outlined. In therian mammals, beetles, many flies and some fish, males are heterogametic (XY) and females are homogametic (XX). In contrast, in birds, snakes, butterflies and some other fish, females are heterogametic (ZW) and males are homogametic (ZZ) (Graves, 2008; Bachtrog et al., 2014; Stöck et al., 2021). Visual identification of the sex based on morphology or behaviour can be challenging or even impossible (Fairbairn et al., 2007), for example for species without sexual dimorphism (Kocijan et al., 2011), but also for juveniles (Kocijan et al., 2011), ancient or historical samples (Buonasera et al., 2020), forensic samples (e.g. hair, Madel et al., 2016), as well as samples of unknown origin, e.g. environmental samples or faeces (Peppin et al., 2010; Sastre et al., 2009).

Molecular sexing through genetic data is an alternative to sexing from observations as it is accurate, simple, cheap, time-efficient, applicable to any tissue and can often piggyback on other genetic analyses (Buonasera et al., 2020). For example, molecular sexing from next-generation sequencing data exploits the difference in sex chromosome ploidy between males and females: in XY-systems, roughly half as many reads are expected to map to the X-chromosome in males than females, and no reads are expected to map to the Y-chromosome in females (Palmer et al., 2019). The same logic applies to ZW-systems. These unique mapping patterns can be used i) to deduce the genetic sex of individuals (sexing), ii) to deduce the type (i.e. autosomal or sex-linked) of scaffolds or iii) to deduce both jointly in case both are unknown. In the following, we describe these three major scenarios.

Current methods to sex individuals assume a reference genome of which the scaffold types are known. A typical case is the sexing of ancient human samples, for which two main methods are currently used: R_y and R_x. R_y (Skoglund et al., 2013) calculates the fraction of reads mapping to the Y-chromosome relative to the total number of reads mapping to both sex chromosomes (X and Y). R_x (Mittnik et al., 2016) calculates the average of the fraction of reads mapping to the X chromosome relative to the number of reads mapping to each autosome. Both methods then assign the sex based on thresholds determined on small data sets (30 and 20 samples, respectively). Nonetheless, most current studies (e.g. Allentoft et al., 2015; Scheib et al., 2018; Margaryan et al., 2020; Furtwängler et al., 2020; Lipson et al., 2022; Liu et al., 2022b; Reitsema et al., 2022) use the same thresholds for sexing without considering differences in sequencing methods, sequencing depth or sample treatment.

The methods R_y and R_x are also both limited to the euploid karyotypes XY and XX, although sex karyotypes aneuploidies in humans are relatively common with an estimated incidence of 1 in 440 births (Breman and Stankiewicz, 2021). Several studies that used large cohorts detected aneuploid individuals, but only by manually inspecting individuals with ambiguous mapping statistics (Rivollat et al., 2020; Villalba-Mouco et al., 2021; Ebenesersdóttir et al., 2018). To our knowledge, there exists currently only a single method that can identify sex aneuploidy in humans (`seGMM`, Liu et al., 2022a), and it does so based on the mean and standard deviation of normalized mapping counts to X and Y using predefined cutoffs.

In the second scenario, the goal is to infer the scaffold types, assuming that the genetic sex of the individuals is known. Scaffold types are often unknown for non-model organisms with low-quality and fragmented draft genome assemblies due to high cost and challenges associated with complete genome assembly (Ellegren, 2014). Approaches to identify sex-linked scaffolds include synteny-based alignments to closely related species with a chromosomal-level genome assembly (Grabherr et al., 2010) or comparison of the sequencing depth of each scaffold between males and females (Palmer et al., 2019). The tool `findZX` (Sigeman et al., 2022) implements a pipeline to identify X/Z chromosomes through differences in sequencing depth and heterozygosity. The method `discoverY` identifies Y-linked scaffolds through differences in sequence similarity and sequencing depth between males and females (Rangavittal et al., 2019).

In the third scenario, both the sex karyotypes of individuals as well as the scaffold types are unknown, as is often case for non-model organisms. The method `SATC` (Nursyifa et al., 2022) infers both sex and sex-linked scaffold in a two step procedure. It first conducts a principal component analysis (PCA) on normalized depth and uses Gaussian mixture clustering to identify males and females. It then uses a t-test to identify sex-linked scaffolds as those for which the normalized depth differs between the two sex groups. However, `SATC` relies on hard thresholds and can not identify Y-linked scaffolds. The method

`SeXY` (Cabrera et al., 2022) uses a synteny-based approach to identify sex-linked scaffolds and performs sexing based on the X to autosome depth ratios. While `SeXY` can perform sexing with a single individual at low depth and was tailored to also work with reference genomes of divergent taxa, its sexing is based on arbitrary thresholds. Other methods (Robledo-Ruiz et al., 2023; Gautier, 2014) can also infer both sex and sex-linked loci but are designed for SNP data sets (e.g. RAD sequencing) and classify each locus individually. None of the tools allows for aneuploid sex karyotypes.

Here, we present a Bayesian method designed to work in all three scenarios described above. Our method, which we call *Bayesian estimation of X and Y*, or `BeXY` for short, jointly infers the type of all scaffolds as well as the sex karyotype for all individuals. In contrast to existing methods, `BeXY` i) does not rely on hard thresholds but instead infers probabilities for scaffold types and sex karyotypes, ii) is flexible in respect of incorporation of existing knowledge on sex-linked scaffolds and sex karyotypes in the model, iii) can identify both X- and Y-linked scaffolds, iv) can detect aneuploid sex karyotypes and v) retains high accuracy for low-depth and highly fragmented genome assemblies. To illustrate our method, we applied it to whole-genome shotgun and target enrichment sequencing data of ancient humans as well as to data from several non-model organisms. Using simulations, we show that our method outperforms current methods in all scenarios.

3 Materials and Methods

3.1 The model

For readability, we will present the model using the XY notation, although the model applies equally well to ZW-systems.

Consider a reference genome consisting of C scaffolds. Let n_{rc} denote the number of sequencing reads of sequencing run $r = 1, \ldots, R$ mapped to scaffold c. These mapping statistics can be obtained easily from indexed `BAM` files either with the `idxstats` command in `Samtools` (Li et al., 2009) or with the `BAMDiagnostics` command in `ATLAS` (Link et al., 2017). Each sequencing run $r = (i, m)$ corresponds to a certain individual $i = 1, \ldots, I$ sequenced with a specific method $m = 1, \ldots, M$ such as, for instance, target enrichment sequencing, whole-genome shotgun sequencing or restriction site associated DNA sequencing (RAD). These methods likely differ in their mapping characteristics and are thus modeled with their own set of parameters. Individuals may have been sequenced with multiple runs and potentially different sequencing methods and the model described here allows to make use of all that data jointly.

We model the vector of counts $\boldsymbol{n}_r = (n_{r1}, \ldots, n_{rC})$ of sequencing run r using a Dirichlet-Multinomial (DM) distribution (Johnson et al., 1997) as

$$\boldsymbol{n}_r \sim \mathrm{DM}\left(N_r, \boldsymbol{\alpha}\left(r\right)\right).$$

Here, $N_r = \sum_c n_{rc}$ is the total number of mapped reads of run r and the parameter vector $\boldsymbol{\alpha}\left(r\right) = \left(\alpha_1\left(r\right), \ldots, \alpha_C\left(r\right)\right)$ models both the expected number and the variance of reads mapping to each scaffold.

We model n_{rc} of run $r = (i, m)$ using two major components: the ploidy p_{ic} of scaffold c in individual i, and some method- and scaffold-specific mapping attractor γ_{mc}. These mapping attractors may differ among scaffolds due to, for instance, scaffold length or mappability as more reads are expected to map to longer scaffolds or to scaffolds with fewer repetitive regions. We model $\alpha_c(r)$ as

$$\alpha_c(r = (i, m)) = \frac{1}{\sigma_m}\gamma_{mc}(p_{ic} + \epsilon_i).$$

Here, ϵ_i is an individual-specific parameter that allows for noise due to erroneously mapped reads and σ_m is a positive scaling parameter that models the variance of the Dirichlet-Multinomial distribution. To avoid non-identifiability issues with σ_m, we impose the constraint that $\sum_{c=1}^{C} \gamma_{mc} = 1$.

Ploidy The ploidy p_{ic} depends on two factors: the sex karyotype s_i of individual i and the scaffold type, i.e. whether a scaffold is autosomal, X or Y-linked. We distinguish seven sex karyotypes, which correspond to the most frequent sex karyotypes observed in humans: the heterogametic sex ($s_i = \mathrm{XY}$), the homogametic sex ($s_i = \mathrm{XX}$), the monosomy $s_i = \mathrm{X0}$ as well as the three trisomies $s_i = \mathrm{XXY}$, $s_i = \mathrm{XYY}$ and $s_i = \mathrm{XXX}$ and the tetrasomy $s_i = \mathrm{XXYY}$.

In theory, the ploidy is given by the number of copies of each scaffold, for example $p_{ic} = 2$ for all autosomal scaffolds or $p_{ic} = 1$ for both X and Y-linked scaffolds of an individual with sex karyotype XY. However, in low-quality reference genomes, the ploidy ratio between the sex karyotypes often differs from the canonical cases due to noise (Nursyifa et al., 2022). We here model the ploidy using a continuous, scaffold-specific parameter $\rho_c \in [0, 1]$ such that $\rho_c = 0$ corresponds to a canonical Y, $\rho_c = \frac{1}{2}$ to a canonical autosome, and $\rho_c = 1$ to a canonical X. Intermediate values of ρ_c then signify ploidies that are different from these cases. The resulting ploidies p_{ic} for all considered sex karyotypes are given in Table 1 and illustrated in Figure 1.

`BeXY` classifies each scaffold into one of four categories: autosomal ($t_c = \mathrm{A}$), X-linked ($t_c = \mathrm{X}$), Y-linked ($t_c = \mathrm{Y}$) or "different" ($t_c = \mathrm{D}$) for ploidies that differ from the canonical cases. We achieve this by modeling ρ_c through a mixture of four Beta distributions. For autosomes, we use a symmetrical Beta distribution $\rho_c | t_c = \mathrm{A} \sim \mathrm{Beta}(\mu, \mu)$, which has an expected value $\mathbb{E}(\rho_c) = \frac{1}{2}$. For X-linked scaffolds, we use a left-skewed distribution $\rho_c | t_c = \mathrm{X} \sim \mathrm{Beta}(\alpha, 1)$ with $\alpha < 1$, which has an expected value close to one. For Y-linked scaffolds, analogously, we use a right-skewed distribution $\rho_c | t_c = \mathrm{Y} \sim \mathrm{Beta}(1, \beta)$ with

Table 1: Ploidies p_{ic} as a function of ploidy parameter ρ_c for all sex karyotypes.

Sex karyotype s_i	$0 \leq \rho_c \leq 0.5$	$0.5 < \rho_c \leq 1$
XY	$1 + 2\rho_c$	$3 - 2\rho_c$
XX	$4\rho_c$	2
X0	$4\rho_c$	$3 - 2\rho_c$
XXY	$1 + 2\rho_c$	2
XYY	2	$3 - 2\rho_c$
XXX	$4\rho_c$	$1 + 2\rho_c$
XXYY	2	2

$\beta < 1$, which has an expected value close to zero. Finally, for non-canonical scaffolds of type $t_c = \mathrm{D}$ we use an uninformative Beta distribution $\rho_c | t_c = \mathrm{D} \sim \mathrm{Beta}(1, 1)$ (see the Supplementary Materials for a more detailed description of the parametrization).

Distribution of karyotypes We assume that the distribution of sex karyotypes s_i in a sample are described well by two parameters, the frequency a of aneuploids and the frequency f of XX to XY karyotypes among euploids, such that

$$\mathbb{P}(s_i | a, f) = \begin{cases} (1-a)(1-f) & \text{if } s_i = \mathrm{XY} \\ (1-a)f & \text{if } s_i = \mathrm{XX} \\ \frac{a}{5} & \text{if } s_i = \mathrm{X0} \text{ or XXY or XYY or XXX or XXYY.} \end{cases}$$

See the Supplementary Material for a description of all prior distributions used.

3.2 Bayesian inference

We use a Markov chain Monte Carlo (MCMC) scheme to generate samples from the posterior $\mathbb{P}(\boldsymbol{s}, \boldsymbol{\rho}, \boldsymbol{\gamma}, \boldsymbol{\epsilon}, \boldsymbol{\sigma} | \boldsymbol{N})$, where $\boldsymbol{N}$ denotes the $R \times C$ matrix of read counts with entries $[\boldsymbol{N}]_{rc} = n_{rc}$. We update t_c using Gibbs sampling and all other parameter using standard Metropolis-Hastings updates (see Supplementary Material).

3.3 Implementation

`BeXY` is a command-line tool implemented in `C++` making use of the MCMC framework of the statistical library `stattools` (bitbucket.org/wegmannlab/stattools). It implements two tasks, `infer` and `sex`. The task `infer` is used to infer sex karyotypes and scaffold types jointly, while the task `sex` is used to only infer sex karyotypes, i.e. only the individual-specific parameters s_i and ϵ_i while using estimates of $\boldsymbol{\rho}$, $\boldsymbol{\gamma}_m$ and σ_m previously learned from a larger data set. Currently, `BeXY` provides such estimates for the human reference genome for both whole-genome shotgun sequencing and 1240K target enrichment capture

protocols. The output of `BeXY` can be loaded into an R-package that visualizes the results and provides an easy interface to classify individuals based on custom thresholds on the posterior probabilities of the sex karyotypes.

3.4 Application to empirical data sets

Ancient humans We downloaded publicly available data of ancient humans sequenced with two different methods. We first downloaded 954 individuals sequenced with Illumina whole-genome shotgun (WGS) sequencing (Antonio et al., 2019; Damgaard et al., 2018; Ebenesersdóttir et al., 2018; Lazaridis et al., 2014; Scheib et al., 2018; Hofmanová et al., 2016; Broushaki et al., 2016; Gamba et al., 2014; Günther et al., 2018; Margaryan et al., 2020; De Barros Damgaard et al., 2018; Marchi et al., 2022; Jones et al., 2017, 2015) in fastq-format and processed these with the `Gaia` part of the `ATLAS`-pipeline (bitbucket.org/wegmannlab/atlas-pipeline). Specifically, we trimmed the reads using `Trim Galore` (github.com/FelixKrueger/TrimGalore) with no quality filter and a length filter of 30. Reads were subsequently aligned to the GRCh38 human reference genome (NCBI RefSeq assembly GCF_000001405.26) using `BWA-MEM` (Li, 2013). Reads with a mapping quality below 30 were filtered out with `Samtools` (Li et al., 2009). Unmapped and, in the case of paired-end sequencing data, unpaired reads were removed with `Samtools`. Samples consisting of multiple libraries were merged using `Samtools`. Duplicate reads were marked using `picard-tools` MarkDuplicates (broadinstitute.github.io/picard). We further downloaded 2,359 individuals sequenced with 1240k target enrichment capture sequencing (Fernandes et al., 2020; Fowler et al., 2022; Fu et al., 2016; Harney et al., 2021, 2022; Kennett et al., 2022; Lazaridis et al., 2022, 2016, 2017; Lipson et al., 2017, 2022; Liu et al., 2022b; Mathieson et al., 2015, 2018; Narasimhan et al., 2019; Novak et al., 2021; Olalde et al., 2018, 2019; Patterson et al., 2022; Prendergast et al., 2019; Reitsema et al., 2022; Rivollat et al., 2020; Sirak et al., 2021; Tiesler et al., 2022; Villalba-Mouco et al., 2021) directly in BAM-format. For both data sets, we used the task `BAMDiagnostics` in `ATLAS` (Link et al., 2017) to count the number of reads that aligned to each chromosome, ignoring duplicates and reads with a mapping quality below 30. The resulting files were used as an input for `BeXY`.

Non-model organisms We ran `BeXY` on the six WGS data sets of varying assembly quality provided by Nursyifa et al. (2022): impala (*Aepyceros melampus*), muskox (*Ovibos moschatus*), waterbuck (*Kobus ellipsiprymnus*), grey whale (*Eschrichtius robustus*), leopard (*Panthera pardus*) and the Darwin's finches species complex encompassing 15 species. We used the `idxstats` files provided by (Nursyifa et al., 2022) and ran `BeXY` and `SATC` on these data sets. We used the default filters of `SATC` for both `BeXY` and `SATC`, i.e. we removed scaffolds with < 100kb length and a normalized depth outside the range $(0.3, 2)$.

3.5 Downsampling experiments

The performance of `BeXY` likely depends on the sequencing depth of the individuals and, in case scaffold types are inferred, also on the sample size, the sex ratio among the samples, and the quality of the reference genome assembly. We tested the limits of `BeXY` and competing methods with respect to each of these challenging factors using dedicated downsampling experiments based on a subset of the ancient WGS data set consisting of all individuals with a depth $> 2\times$ ($n = 116$) that were also all identified as euploid on the full data sets.

To evaluate the robustness of sexing individuals with respect to low-depth data of euploid individuals, we downsampled (with replacement) the read counts of all individuals in the subset to various depth ranging from 200,000 to 100 reads per individual. We ran `BeXY` with the task `sex` on 100 replicates of downsampled counts, and set the prior parameters on s to the values expected for a human data set, $f = \frac{1}{2}$ and $a = \frac{1}{440}$ (Breman and Stankiewicz, 2021). We considered all classifications with state posterior probabilities $\mathbb{P}(s_i|\boldsymbol{n}_r) > 0.9$ as confident. We then compared the sex inferred from the downsampled data set with that inferred from the full data set. We also ran the two competing sexing methods R_x (Mittnik et al., 2016) and R_y (Skoglund et al., 2013) on the same data set using default parameters and counted the classifications "Sex assignment: The sample could not be assigned" (for R_x) as well as "Not Assigned" (R_y) as uncertain and all others as confident.

To investigate the power of the task `sex` to detect aneuploid individuals at various depths, we simulated aneuploid individuals based on the samples in the subset. We used individuals with karyotype XY to simulate samples with karyotypes XXY, XYY and XXYY and individuals with karyotype XX to simulate samples with karyotypes X0 and XXX as follows: Let f_x and f_y denote the the relative change in ploidies between the karyotype to simulate and the karyotype of the individual serving as template (e.g. $f_X = 2, f_Y = 2$ to simulate the karyotype XXYY from an XY individual, or $f_X = 0.5, f_Y = 1$ to simulate a karyotype X from an XX individual). We then sampled reads (with replacement) from the vector $\boldsymbol{n}_r$ according to the probabilities

$$p_{rc} = \frac{1}{N_r + (f_x - 1)n_{rx} + (f_y - 1)n_{ry}} \begin{cases} n_{rc} & \text{if } c \in \{1, \ldots, 22\} \\ f_x n_{rx} & \text{if } c = X \\ f_x n_{ry} & \text{if } c = Y. \end{cases}$$

Using this procedure, we simulated samples for various depth ranging from 200,000 down to 100 reads per sample. We ran `BeXY` with the task `sex` on 100 replicates of downsampled counts and identified confident classifications as described above, both on a per-karyotype basis as well as for euploid and aneuploid karyotype classes, ensuring that all karyotype were equally frequent within a class. Since the

prior on the number of aneuploid samples, a, has a large impact on the classification accuracy of euploid and aneuploid individuals, we ran `BeXY` with both $a = \frac{1}{440}$ and $a = \frac{1}{2}$. We ran the competing sexing method `seGMM` (Liu et al., 2022a) on the same data set. Since the available implementation of `seGMM` requires a VCF file to perform the clustering of the samples for subsequent sexing, we re-implemented their approach and calculated the relevant statistics needed for sexing (the mean and standard deviation of normalized read counts for the X and Y chromosome for XX and XY samples) based on the full counts. While re-implementing, we also fixed an obvious bug in their code that led to a misclassification of XXX individuals (XXX karyotypes are expected to have 1.5 times the number of reads mapping to the X-chromosome than XX karyotypes, not twice as many). We counted all classifications that could not be assigned as uncertain and all others as confident.

We also investigated the power to infer sex karyotypes scaffold types jointly, using the same downsampled counts as for the first downsampling experiment described above (only euploids). We ran `BeXY` with the task `infer` and identified confident classifications as described above. We ran the competing method `SATC` (Nursyifa et al., 2022) on the same data set. For comparability with `BeXY`, we turned off the normalized depth range filter by setting it to $(0, 100)$ in `SATC`. `SATC` does not provide an estimate of classification uncertainty and we assumed all classifications as confident. But we note that `SATC` throws an error if no good candidates for sex scaffolds were found through clustering or when one of the inferred sex groups only had one member. We counted these cases as uncertain.

To evaluate the robustness of our method to small data sets or those with large sex ratio imbalances when jointly inferring sex karyotypes and scaffold types, we first downsampled all individuals in the subset to 20K reads and then randomly compiled data sets with various XY:XX proportions differing in size and imbalance. We ran `BeXY` and `SATC` as describes above.

To evaluate the robustness of our method with respect to low-quality reference genome assemblies, we simulated a low-quality reference genome based on the human reference genome GRCh38. To match the roughly exponential distribution of empirical low-quality reference genomes (e.g. waterbuck from Nursyifa et al., 2022), we defined length categories of 10^6, $5 \cdot 10^5$, $2 \cdot 10^5$, 10^5, $5 \cdot 10^4$, $2 \cdot 10^4$ and 10^4 and split the chromosomes into scaffolds of these lengths. To limit mapping issues, we excluded the telomeres (15kb at each end of each chromosome), the pseudo-autosomal regions (PAR) on the X and Y chromosome as well as the centromeres of all chromosomes (all downloaded from ncbi.nlm.nih.gov/grc/human), with an additional buffer of 100kb around each of these regions. After splitting, we further excluded all resulting scaffolds that overlapped by more than 90% with gap regions consisting of N (hgdownload.soe.ucsc.edu/goldenPath/hg38/database/gap.txt.gz, Nassar et al., 2023) as well as all scaffolds that overlapped by more than 90% with regions listed in the ENCODE Blacklist (Amemiya et al., 2019). We then re-mapped the sequencing data of all samples against this artificially created low-quality reference

genome. To reduce computational time, we downsampled each `fastq` file to 10 millions reads using the sample option in `seqtk` v1.4 (github.com/lh3/seqtk) prior to mapping. We then downsampled read counts to 1,000,000 reads per sample with replacement for 50 replicates and ran `BeXY` with the task `infer` and `SATC`. The performance of `SATC` turned out to be rather poor when no depth filter was used, we ran it only on scaffolds that had a normalized depth in the interval $(0.3, 2)$, which corresponds to their default filter. For `BeXY`, we only excluded scaffolds that had zero counts for all samples, as these are not identifiable. We considered all scaffold type classifications by `BeXY` as confident if their state posterior probabilities $\mathbb{P}(t_c|\boldsymbol{n}_r) > 0.9$. `SATC` does not provide an estimate of uncertainty in classifying scaffold types and we assumed all classifications as confident. But as above, we considered all classifications as uncertain if `SATC` threw an error. We counted all scaffolds that were removed by the filter as a uncertain classification.

4 Results

4.1 Downsampling experiments

Sexing for low-depth data We first investigated the robustness of `BeXY` to low-depth sequencing data of euploid individuals and compared its performance to that of R_x and R_y. As shown in Figure 2A, `BeXY` correctly assigned the sex karyotype with high confidence to $> 97\%$ of all samples even at only $1,000$ reads per sample and to 24% at 100 reads per sample, with a low false discovery rate (FDR) of around 3% at very low depth. The second most powerful methods was R_x, which confidently classified a similar fraction of all samples correctly, albeit with a much elevated rate of misclassifications, which results in a FDR of up to 26% at 100 reads per sample. In contrast, R_y confidently classified much fewer samples correctly at an even much higher FDR of up to 55%.

We then evaluated the power of `BeXY` to detect aneuploid sex karyotypes at low-depth data, and compared it to the competing method `seGMM`. We first evaluated the power of `BeXY` using a relaxed prior on the number of aneuploid individuals of $a = \frac{1}{2}$. As shown in Figure 2B, `BeXY` correctly assigned both euploid and aneuploid sex karyotypes with high confidence to $> 90\%$ of all samples at 20,000 reads. We note that the classification accuracy of the euploid karyotypes XY and XX is lower than in Figure 2A, because the prior does not favour euploid karyotypes anymore at noisy counts. `seGMM` correctly assigned $> 80\%$ of euploid samples and only 47% of aneuploid samples at 20,000 reads. For lower read counts, `seGMM` has a higher power, but also a much elevated FDR of up to 80% for euploid samples and 50% for aneuploid samples.

In Figures 2C and D we show the performance for each karyotype individually. As shown in Figure 2C, the karyotypes that contain a Y chromosome (XY, XXY, XYY and XXYY) require around 20,000

reads to be classified with an accuracy of $> 80\%$ by `BeXY`, while the karyotypes that contain only X chromosomes (X0, XX and XXX) require only 2,000 reads to obtain the same accuracy. This is explained by the short length of the Y chromosome, which results in small and rather noisy counts. As shown in Figure 2D, classification accuracy of `seGMM` was very variable across karyotypes, with the euploid karyotypes and XXY having a classification accuracy similar to that of `BeXY` and the karyotypes X0, XXX and XYY having low classification accuracy regardless of depth. At low depth, many karyotypes suffered from high misclassification rates, likely because the hard thresholds become unreliable.

When sexing individuals at low depth, the choice of the prior probability on an individual to be aneuploid may have a considerable impact on the performance of `BeXY`. To investigate this we repeated the above inference also with a stringent prior of $a = \frac{1}{440}$, which is strongly favoring euploid genotypes. As a consequence, and as also seen in Figure 2A, euploid karyotypes required fewer reads (about 1,000) to be classified correctly, while aneuploid karyotypes were wrongly classified as euploids (X0 and XXX as XX and XXY, XYY and XXYY as XY) at low depth (Supplementary Figure 1).

Joint inference for low-depth data We next compared the performance of `BeXY` to `SATC` in inferring sex karyotypes jointly with scaffold types. We first investigated the impact of sequencing depth using the full subset of 116 samples. If samples had $> 1,000$ reads, both methods performed well and correctly assigned the sex karyotype with high confidence to $> 99\%$ and $> 95\%$ of all samples respectively (Figure 3A). With lower depth, both methods lose power, but differed in that `BeXY` rarely confidently misclassified individuals while median misclassification rate for `SATC` was up to almost 40%.

We next investigated the impact of small sample sizes, imbalanced sex ratios, or both. In 97% and 68% of all simulations conducted, all samples were correctly classified by `BeXY` and `SATC`, respectively. In 27% and 87% of all cases with misclassifications, `BeXY` and `SATC` misclassified all samples to the opposite sex karyotype, suggesting that the challenge at low sample number or with imbalance sex ratios lies in correctly identifying sex-linked scaffolds. As shown in Figure 3B, the fraction of replicates in which all individuals were correctly classified was generally higher for `BeXY` than `SATC`, especially for data set with imbalanced sex ratios. If a sex karyotype was represented by only two individuals, for instance, `SATC` classified all samples correctly in less than 20% of the replicates. In comparison, `BeXY` still classified all samples correctly in 99% of the replicates under these conditions. Notably, `BeXY` also classified all samples correctly in more than 84% of the replicates if a sex karyotype was represented by a single individual - a case in which `SATC` does not attempt any classification.

Low-quality reference genome assemblies We investigated the power of `BeXY` to identify scaffold types in the case of low-quality reference genome assemblies. The simulated reference genome consisted of

10,574 scaffolds ranging from 10^6 to 10^4 bases in length. As shown in Figure 3C, the median classification accuracy of `BeXY` was $> 99\%$ for all larger scaffolds, but dropped slightly to about 95% and 89% for the smallest X-linked and Y-linked scaffolds simulated, respectively. `SATC` had similar power to identify autosomes (median of $> 98\%$ for all scaffold lengths), although it interestingly misclassified a few long scaffolds as abnormally sex-linked in some replicates. While `SATC` does not classify Y-linked scaffolds, the median classification accuracy for X-linked scaffolds was around 85% for long scaffolds and dropped to 24% for short scaffolds of 10kb, albeit high variability between replicates.

4.2 Application to human data set

We analyzed 954 ancient human samples sequenced with whole-genome shotgun sequencing and 2,359 ancient human samples sequenced with the 1240k capture target enrichment technique. In the whole-genome data set, visualized in Figure 4AC, all individuals were classified with high confidence as XY ($n = 608$) or XX ($n = 345$) except YGS-B2, which was classified as XXY with 100% posterior probability, in line with earlier reports (Ebenesersdóttir et al., 2018). In the 1240k capture data set, visualized in Figure 4BD, we identified 1,101 samples as XX and 1,249 samples as XY with high confidence (posterior probability for the given karyotype larger than 90%). We confirmed the karyotype of all three samples that were previously classified as aneuploid: ALM062.A0101.TF1.1 (XXX, Villalba-Mouco et al., 2021), CLL011.A0101.TF1.1 (XXY, Villalba-Mouco et al., 2021) and HBS006 (XXY, Rivollat et al., 2020) with very high confidence (100% posterior probability for the given karyotype). In addition, we identified six novel aneuploid samples with high confidence: I12935 (XYY, 100%), I18427 (XXY, 100%), I20769 (XXY, 99.8%), I19361 (XXY, 99.9%), I19480 (XXY, 96.9%), and I18426 (XXX, 86.7%). For two samples, the karyotype remains unclear: I12437 had a posterior probability of 34.1% to be XYY and 65.9% to be XY and I20754 had a posterior probability of 60% to be XX and 40% to be XY. This last sample had the lowest read count of all samples (6,983 reads) and was previously reported as being likely contaminated (Harney et al., 2021), which may explain its uncertain sex karyotype assignment.

4.3 Application to data sets from non-model organisms

We ran `BeXY` on the six mammal and bird WGS data sets provided by (Nursyifa et al., 2022) and compared the sex and scaffold-type assignment to that obtained with `SATC`. For all data sets, the two methods did not differ in the sex karyotype classifications. Both methods also mostly agreed in their scaffold-type assignments and classified on average 94.9% of all scaffold identically across data sets. The most common difference in scaffold-type was that `SATC` classified a scaffold as "abnormally sex-linked" while `BeXY` classified it as autosomal or X-linked. Notably, and while `SATC` cannot detect Y/W-linked

scaffolds, `BeXY` found clear evidence of Y/W-linked scaffolds in muskox, waterbuck and finches, which `SATC` labelled as "abnormally sex linked". Specifically, `BeXY` classified the scaffolds 213 and 46825 in muskox, the scaffolds 1604, 2519, 3205, 3507, 3814, 4043, 4087, 4219, 4352 and 4785 in waterbuck as well as the scaffolds NW_005054701.1, NW_005054702.1, NW_005054736.1, NW_005054753.1, NW_005054754.1 in finches with a 100% posterior probability as being Y/W-linked.

5 Discussion

With the rapid growth of population genomics studies for non-model organisms and ancient samples, there is a need for accurate sex karyotype and scaffold type assignment for low-depth samples. We here present `BeXY`, a Bayesian method that jointly infers sex karyotypes and identifies sex-linked scaffolds from mapping statistics. Our method is flexible with respect to existing knowledge (e.g. scaffold types), retains high accuracy for low-depth samples, is robust to small data sets or those with highly imbalanced sex ratios, and outperforms all existing methods in the field.

One use case of `BeXY` are non-model organisms, for which both the genetic sex of individuals as well as scaffold types are unknown. `BeXY` only requires mapping statistics, namely the number of reads mapping to each scaffold as well as scaffold lengths, and does not require any prior knowledge on sex karyotypes or sex-linked scaffolds. Importantly, `BeXY` introduces a continuous parametrization of the expected ploidy. This allows for the accommodation of noisy scaffolds showing aberrant ploidy ratios due to mapping issues or mistakes in the assembly, which was previously shown to complicate the identification of sex-linked scaffolds (Nursyifa et al., 2022). Using simulations, we showed that `BeXY` has much higher power to accurately infer scaffold types than the competing methods `SATC` (Nursyifa et al., 2022), particularly for data sets with only few individuals or highly imbalanced sex ratios, also for low-depth samples ($1,000$ reads per sample). In contrast to `SATC`, `BeXY` also accurately identifies Y-linked scaffolds, which `SATC` puts in the same class as those with aberrant ploidy ratios.

The second major use case of `BeXY` is the genetic sexing of (single) individuals, for example for ancient humans. For this scenario, `BeXY` requires mapping statistics of the individual, along with estimates of parameters $\boldsymbol{\rho}$, $\boldsymbol{\gamma}_m$ and σ_m that have been inferred on a larger data set. It is important that these parameters were inferred not only from a data set of the same species, but also from data produced with the same sequencing method, as different methods may differ in their per-scaffold expected read counts. In the case of ancient human samples, for instance, whole-genome shotgun and target enrichment capture data differs considerably in their distribution of reads across chromosomes, as the former is mainly influenced by the length of each chromosome, and the latter mainly by the number of sites targeted per chromosome. While `BeXY` already provides suitable parameter estimates for humans for both of these

sequencing methods, they would have to be re-estimated for a different species or a new capture technique, for instance.

Genetically sexing individuals with `BeXY` has several advantages over existing methods such as R_x, R_y, `SATC` and `seGMM`. First, `BeXY` is a Bayesian method that calculates the posterior probabilities for each sex karyotype, whereas all existing methods that do sex assignment from read count data are based on hard thresholds. As we showed with simulations, the use of hard thresholds is problematic in the case low-depth samples, which were often un- or misclassified. Second, `BeXY` accurately identifies aneuploid sex karyotypes, which are expected to be found in most large data sets. There exists currently only a single method, `seGMM`, that identifies aneuploid karyotypes from read counts, and as we showed with simulations, `BeXY` has considerably higher power to accurately infer such karyotypes, only requiring about 20,000 reads per individual in the case of ancient human samples.

Since `BeXY` is a Bayesian method, the choice of the prior may have a big influence if data is limited. That is particularly true for a, the expected fraction of aneuploids in the sample. Our simulations suggest that if a small value of a was used, such as the known distribution of aneuploids in the human population $a = \frac{1}{440}$, euploid individuals should be accurately sexed with as few as 1,000 reads, but aneuploid individuals with that little data would often be confidently misclassified as euploids as the data can not overcome the prior. If a larger value of $a = \frac{1}{2}$ was used, our simulations indicate that both euploids as well as aneuploids individuals should hardly ever be misclassified, but that about 20,000 reads per sample are required for confident classification. We therefore suggest to use a large value of $a = \frac{1}{2}$ for samples with more than 20,000 reads, but to restrict the analysis to euploid karyotypes with a more stringent prior if fewer reads are available. In the ancient human data sets analyzed in this paper, only 1.4% of all individuals had less than 20,000 reads, therefore $a = \frac{1}{2}$ is expected to result in an accurate classification of sex karyotype for the majority of individuals.

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7 Data Accessibility and Benefit-Sharing

`BeXY` is freely available through the `git`-repository at bitbucket.org/wegmannlab/bexy. All scripts and data sets (input files) used here are available at https://doi.org/10.5281/zenodo.8344992, where we also provide a complete table of all previously published sequencing data used.

8 Author Contributions

The work was conceived and the research designed by Madleina Caduff, Christoph Leuenberger and Daniel Wegmann. The data was analysed by Raphael Eckel and Madleina Caduff. Madleina Caduff and Daniel Wegmann wrote the manuscript with input from Raphael Eckel. All authors read and approved the final version of the manuscript.

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10 Tables and Figures

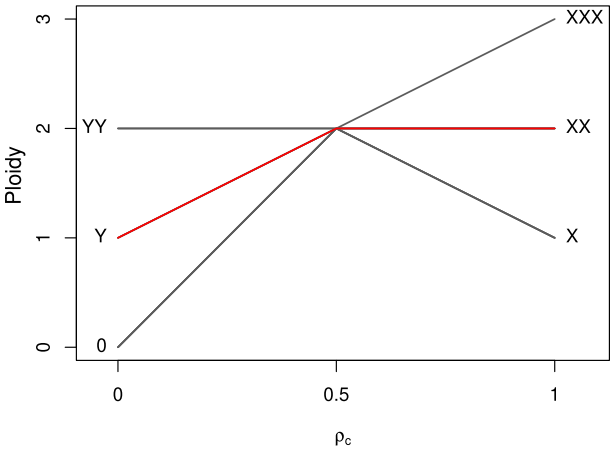

Figure 1: Ploidy as a function of ρ_c for all sex karyotypes, obtained by following the line from the number of Y copies to the number of X copies of that karyotype. The red line exemplifies the ploidy function for the karyotype XXY.

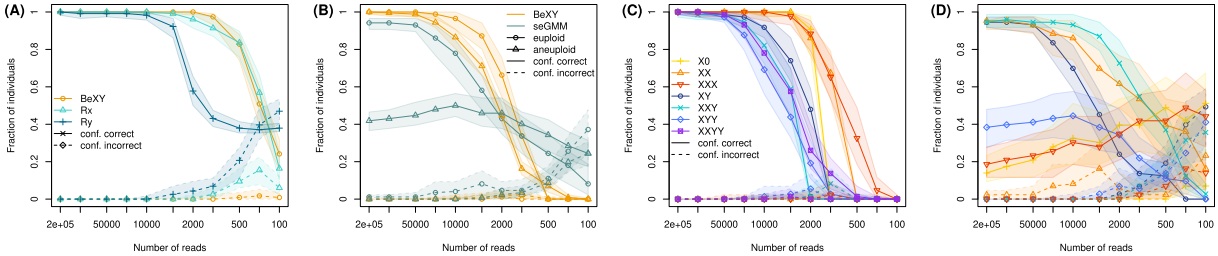

Figure 2: Power to sex individuals. Shown are the median (line with symbols) and 98% confidence interval (shaded area) of the fraction of individuals classified as confidently correct (solid line) and confidently incorrect (dashed line) across 100 downsampling replicates. (A) The performance of `BeXY` compared to R_x and R_y on euploid karyotypes. (B) The power of `BeXY` compared to `seGMM` to classify both euploid and aneuploid karyotypes using a relaxed prior of $a = \frac{1}{2}$. (C) The same results of `BeXY` as in B, but plotted by karyotype. (D) The same results of `seGMM` as in B, but plotted by karyotype.

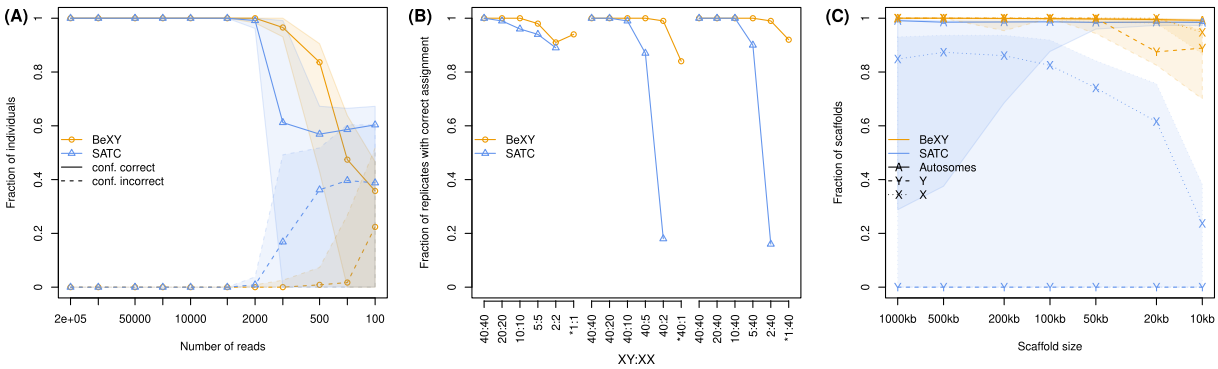

Figure 3: Power to infer sex karyotypes of individuals and scaffold types jointly. (A) The the median (line with symbols) and 98% confidence interval (shaded area) of the fraction of individuals classified as confidently correct (solid line) and confidently incorrect (dashed line) as a function of sequencing depth across 100 downsampling replicates for both `BeXY` and `SATC`. (B) The fraction of replicates where the sex karyotype of all individuals was correctly assigned for small (left) and imbalanced (middle and right) data sets. Note that `SATC` requires at least two individuals per karyotype and could not be evaluated for the data sets marked with an asterisk. (C) The power of `BeXY` and `SATC` to classify scaffold types for low-quality reference genomes. Shown are the median (line with symbols) and 98% confidence interval (shaded area) of the fraction of scaffolds classified as confidently correct as a function of scaffold length across 50 replicates for each scaffold type. Note that `SATC` does not classify Y-linked scaffolds.

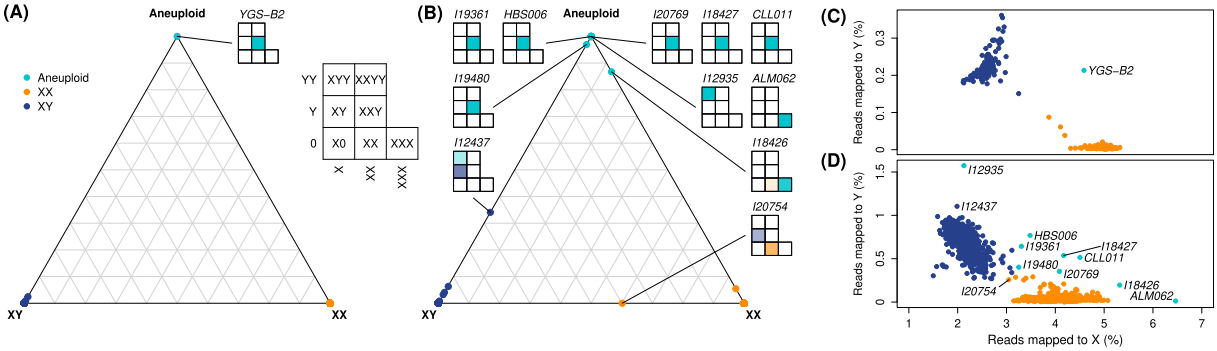

Figure 4: Sex karyotype classification of ancient samples. (A,B) Ternary plot showing the posterior probabilities for each sample to have an XY, XX or aneuploid sex karyotype for the ancient WGS (A) and 1240k target enrichment capture (B) data sets. The color of the points refers to the posterior mode of the sex karyotype. The per karyotype posteriors of all samples identified as aneuploid or with uncertain sex karyotypes are shown using shaded within a 7-cell matrix of the x-axis corresponds to having one, two or three copies of the X-chromosome and the y-axis corresponds to having zero, one or two copies of the Y-chromosome. The samples CLL011.A0101.TF1.1 and ALM062.A0101.TF1.1 were abbreviated to CLL011 and ALM062, respectively. (C,D) Scatter plot for the ancient WGS (C) and 1240k target enrichment capture (D) data sets showing the percentage of reads mapped to Y against the percentage of reads mapped to X, with the same individuals highlighted as in A and B.