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Interpreting Patterns of X Chromosomal Relative to Autosomal Diversity in Aye-Ayes (*Daubentonia madagascariensis*)

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ABSTRACT

We here present high-quality, population-level sequencing data from the X chromosome of the highly-endangered aye-aye, *Daubentonia madagascariensis*. Using both polymorphism- and divergence-based inference approaches, we quantify fine-scale mutation and recombination rate maps, study the demographic and selective processes additionally shaping variation on the X chromosome, and compare these estimates to those recently inferred from the autosomes in this species. Results suggest that an equal sex ratio is most consistent with observed patterns of variation, and that no sex-specific demographic patterns are needed to fit the empirical site frequency spectrum. Further, reduced rates of recombination were observed relative to the autosomes as would be expected, whereas mutation rates were inferred to be similar. Utilizing the estimated population history together with the mutation and recombination rate maps, we evaluated evidence for both recent and recurrent selective sweeps as well as balancing selection across the X chromosome, finding no significant evidence supporting the action of these episodic processes. Overall, these analyses provide new insights into the evolution of the X chromosome in this species, which represents one of the earliest splits in the primate clade.

1 | Introduction

Apart from determining biological sex, sex chromosomes (typically X and Y in mammals) play a fundamental role in the evolution of behavior and development, with genes located on these chromosomes contributing to sex-specific traits and disease susceptibility. Yet, despite their importance, the great majority of studies to date examining primate sex chromosome evolution have focused upon the great apes, or biomedically-relevant species (Makova et al. 2024), leaving large swathes of the primate clade poorly studied in this regard. Bringing these analyses to a strepsirrhine, we here consider the evolutionary history of the X chromosome of the aye-aye (*Daubentonia madagascariensis*), a nocturnal lemur endemic to Madagascar (Ancrenaz et al. 1994; Randimbiharinirina et al. 2019). Aye-ayes are behaviorally and morphologically quite distinctive; compared to other lemurs, they

Susanne P. Pfeifer and Jeffrey D. Jensen jointly supervised the project.

Summary

- The evolutionary forces shaping sex chromosomal relative to autosomal variation have the potential to vary dramatically, and we here provide an in-depth analysis of these factors in a strepsirrhine, the aye-aye (*Daubentonia madagascariensis*).
- We newly identify the pseudoautosomal region (PAR) in the current reference assembly of the species, generate mutation and recombination rate maps across the X chromosome, find evidence supporting roughly equal male:female sex ratios, and investigate X-specific demographic and selective dynamics.
- This study thus provides important insights into this highly endangered species, and will represent a useful point of comparison for future studies of primate sex chromosomes.

have fewer plant associations despite seeds being a sizable portion of their diet (Tonos et al. 2024), and their evolutionary lineage has been suggested to be characterized by lower rates of speciation (Scott 2022). Most relevant to this study, however, are those features pertaining to their sexual characteristics. As lemurs are the largest group of mammals without male-biased sexual size dimorphism, there is little difference between male and female aye-ayes morphologically outside of primary sex characteristics like gonads (Sterling 1993; Kappeler et al. 2019), and in terms of diet and typical behavior, males and females are also largely alike (Ancrenaz et al. 1994).

Previous work has reported little evidence of biased sex ratios at birth or during juvenile development in the wild, though studies in captive aye-ayes described a slight bias towards female offspring in general (approximately 0.71 male:female sex ratio at birth; Zehr et al. 2014), but with a subtle bias towards male offspring from younger sires (Tanaka et al. 2019). Considering that aye-ayes begin reproductive activity around 3.5 to 4 years of age, and remain active through their median longevity of ~25 years (Jones et al. 2009; Zehr et al. 2014), evidence suggests that across the entire population the at-birth sex ratio is likely not strongly different from 1:1. The timing of reproductive events is not seasonally restricted; pregnancy lasts nearly 6 months, with single-births being the norm, and mothers rear their offspring for 1.5 to 2 years (Sterling 1994; Jones et al. 2009; Zehr et al. 2014). Thus, reproduction is generally limited by the extended interbirth interval, thereby reducing the availability of females in estrus that may be anticipated from census size alone. While males of other species have been known to accelerate the initiation of estrus through infanticide, such behavior has only been reported once in aye-ayes (Rakotondrazandry et al. 2021). As such, it is not clear if this single incidence is indicative of a reproductive strategy, an extension of previously observed aggression between older males and younger, but weaned, males, or a result of more general resource guarding (i.e., removing a potential competitor for food rather than motivated by a desire to expedite estrus). Irrespective of the cause, such interactions are presumably rare as the aye-aye is a solitary species known for having sizable, though often overlapping, home ranges (Randimbiharinirina et al. 2019).

Although largely restricted to mature and non-degraded forests (Farris et al. 2011; Miller et al. 2018), aye-ayes are distributed relatively widely across Madagascar. However, there are notable differences in the territorial ranges associated with each sex. Specifically, the earliest estimates of range sizes found that of males to be approximately 5 times larger (~ 40 ha for females, and ~200 ha for males); however, this study was conducted on a small island near Madagascar of only 500 ha in size (Sterling 1993). Later studies on the Madagascar mainland found home ranges for aye-ayes to be much larger, ranging from 800 to 1000 ha for males and 100 ha for females (Randimbiharinirina et al. 2018) to as large as 2586 ha for males and 765 ha for females (Sefczek et al. 2020). Notably, though male ranges were consistently found to be multiple times larger than female ranges, they were additionally described to minimally overlap with one another and heavily overlap with the ranges of several females (Sterling 1993; Ancrenaz et al. 1994). Such sex-based differences in home range size are common in primates, and have been described elsewhere (e.g., Singleton and van Schaik 2001). If these differences in range size and connectivity were to result in fewer reproductively contributing males, this distinction could be expected to reduce male relative to female effective population sizes (Kappeler 2017).

Due to their association with sex determination and sex-associated characteristics, sex chromosomes are likely to be particularly sensitive to these sex-based differences in ecology, and they thus require separate consideration from autosomal chromosomes. For example, because different chromosomes within a genome are generally considered to be effectively unlinked (Morgan 1911), each may be treated as an independent sample of the evolutionary history of the population in question. Following from this, autosomes may be used as pseudo-replicates when performing population genomic analyses (e.g., demographic estimation). However, while the sex chromosomes naturally share their history with autosomes, they are further shaped by factors ranging from the exact form of sex determination, the age of the sex chromosome system, and the population sex ratio, to name but a few contributing factors (for a discussion, see the reviews of Ellegren 2011; Bachtrog et al. 2014).

Mammals are generally characterized by an XY sex-determination system in which the heterogametic sex (XY) develops into males and the homogametic sex (XX) develops into females. Outside of a relatively small pseudoautosomal region (PAR), no recombination occurs between the X and Y chromosomes (unless otherwise stated, discussion of the "X chromosome" in this study is restricted to the larger, non-pseudoautosomal region (non-PAR) most impacted by the unique properties of sex chromosomes). Additionally, oogenesis is typically restricted to a single developmental period, while spermatogenesis occurs throughout the lifespan of an organism. These factors alone could be expected to lead to differences in evolutionarily-important factors including rates of mutation and recombination, particularly as compared to autosomes in which chromosomal copy number is identical between the sexes. For example, there are fewer copies of the X chromosome relative to an autosome (3/4 under even sex ratios), generally leading to a lower effective population size, resulting in differing degrees of genetic drift and modifying demographic scaling on the X (e.g., Pool and Nielsen 2007; Singh et al. 2007).

Relatedly, sex-specific migration may further modify demographic patterns on the X relative to the autosomes (Laporte and Charlesworth 2002). Secondly, sex chromosomes uniquely experience a prolonged portion of their history in a haploid state, meaning that when in males, mutations will be directly visible to selection even whilst rare and regardless of dominance (Haldane 1924; Charlesworth et al. 1987; and see Bachtrog et al. 2009). Moreover, Hartl (1971, 1972) noted that the change in mean fitness at X-linked loci experiencing equivalent selection in both sexes would be greater than for autosomal loci. Thirdly, owing to differences in the number of germ cell divisions in males and females, male-biased mutation patterns have been widely observed (e.g., Haldane 1946; Hurst and Ellegren 1998; McVean 2000).

In primates specifically, numerous insights have been gained with regard to X chromosome evolution, often discussed relative to the expected 3:4 ratio of effective population sizes on the X:autosomes. Notably, if there exists a higher male variance in reproductive success, this ratio may exceed the expected 0.75, reaching as high as 1.125 when there is effectively a single reproducing male; if the variance in female reproductive success is higher, the ratio may approach 0.5625 when there is effectively a single reproducing female (Charlesworth 2001). As might be expected, the genetic diversity on the sex chromosomes of humans have been most heavily studied (see the review of Webster and Wilson Sayres 2016), with results indicating, for example, deviation in this ratio of autosomal to X-linked genetic diversity relative to the 0.75 expectations (e.g., Keinan et al. 2009), as well as likely differences between the historical effective population sizes of males and females (e.g., Wilder 2004). Outside of humans, the X:autosome ratio of diversity in primates has been observed to be particularly low in gorillas and orangutans (Prado-Martinez et al. 2013), but nearer to a neutral, equal sex ratio expectation in Tonkean macaques (Evans et al. 2014).

To investigate the evolutionary dynamics of the X chromosome in the sexually monomorphic aye-aye, we have here obtained high-coverage sequence data of X chromosomes from a 12-individual three-generation pedigree that includes five unrelated founder individuals. Usefully, there have been considerable advances in our understanding of the evolutionary genomics of aye-ayes over the past year, which have here served to greatly improve our resolution of evolutionary processes acting on the X. These developments have included the generation of an annotated chromosome-level aye-aye genome assembly (Versoza and Pfeifer 2024), the recent estimation of a demographic history for this population based on the autosomal site frequency spectrum (SFS; Terbot et al. 2025), genomic scans for evidence of positive and balancing selection across the autosomes (Soni et al. 2025a), as well as high-quality pedigree-based estimates of autosomal mutation (Versoza et al. 2025a) and recombination (Versoza et al. 2025c) rates, and structural variant architecture (Versoza et al. 2025b). More recently, indirect estimates of autosomal mutation and recombination rates have been made, based upon divergence data and patterns of linkage disequilibrium (Soni et al. 2025c), respectively, and the shape of the distribution of fitness effects (DFE) characterizing exonic divergence across the autosomes has additionally been inferred (Soni et al. 2025b). These studies all provide a novel and exceptional framework in which to perform comparative analyses of the evolutionary dynamics acting on the autosomes relative to the, as of yet unexplored, X chromosome in this species.

Thus, we here present analyses investigating the demographic history of the aye-aye X chromosome relative to the autosomal genome, use this demographic history to quantify likely sex ratios in this species, employ forward-in-time simulations to account for the DFE and the resulting background selection effects across the X when model-fitting, estimate fine-scale mutation and recombination rate maps across the chromosome, and perform both polymorphism- and divergence-based scans for functional regions evolving under recurrent positive or balancing selection. In so doing, we also provide the first fine-scale mapping of the PAR of the aye-aye X chromosome. Taken together, this study thus represents the first comprehensive investigation of X chromosome dynamics in this highly endangered primate.

2 | Methods

2.1 | Animal Subjects

This study was approved by the Duke Lemur Center's Research Committee (protocol BS-3-22-6) and Duke University's Institutional Animal Care and Use Committee (protocol A216-20-11), and performed in compliance with all regulations regarding the care and use of captive primates, including the U.S. National Research Council's Guide for the Care and Use of Laboratory Animals, the U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Nonhuman Primates.

2.2 | Population Genomic Data

We obtained high-coverage sequence data of X chromosomes from a 12-individual three-generation pedigree that includes five unrelated founder individuals (three females and two males; Terbot et al. 2025; Versoza et al. 2025a). In brief, following the approach outlined in earlier work (Terbot et al. 2025), we first mapped sequencing reads to the species' genome assembly (GenBank assembly: GCA_044048945.1; Versoza and Pfeifer 2024) using BWA-MEM v.0.7.17 (Li and Durbin 2009) and then called variants using the Genome Analysis Toolkit (GATK) v.4.2.6.1 workflow (van der Auwera and O'Connor 2020). Following developer recommendations, we adjusted the level of heterozygosity to that previously observed in the species (*--heterozygosity 0.0005*; Perry et al. 2013), set the sample ploidy according to the biological sex of each individual (*--sample-ploidy 1* for males and *--sample-ploidy 2* for females), and disabled PCR error correction as the data originated from PCR-free sequencing (*--pcr-indel-model* NONE). To obtain information regarding the sites accessible to our study, we emitted calling information at each nucleotide in the genome (*-ERC BP_RESOLUTION* mode in HaplotypeCaller and *-all-sites* mode in GenotypeGVCFs), though only considered sites in our downstream analyses at which genotype information was available for all individuals. Lastly, to allow for comparison with the autosomes, we followed the methodologies described in earlier work to generate high-quality datasets of putatively neutral regions for the inference of population structure and demography

(Terbot et al. 2025) as well as chromosome-wide data for the inference of recombination and mutation rates (Soni et al. 2025c) and selection (Soni et al. 2025a).

2.3 | Determining the Location of the Pseudoautosomal Boundary

We used differences in male:female coverage to identify the pseudoautosomal boundary separating the non-PAR from the PAR.

2.4 | Estimating the Demographic History of the X Chromosome

Our initial step was to determine if the demographic history previously estimated from the autosomes (Terbot et al. 2025) remained consistent with the X chromosome, or whether a history of sex-specific population dynamics would be necessary to explain observed patterns of variation. As all X chromosomal samples were collected from the "non-North" deme described in Terbot et al. (2025), we anticipated that our sample should similarly appear unstructured. To verify this, the non-PAR region of the X chromosome was analyzed using ADMIXTURE v1.3 (Alexander et al. 2009), allowing for 1 to 5 source demes. The optimal number of demes was determined by finding the deme number that minimized the cross-validation error before increased error in subsequent deme numbers (i.e., the first local minimum cross-validation error).

With a single source deme for the X chromosomes confirmed (see below), the subsequent demographic model only considered the history of the non-North deme. Using SLiM v.4.3 (Haller and Messer 2023), we thus simulated 100 X chromosomes under the demographic history for the non-North deme previously estimated for the autosomes. Briefly, this model entails an ancestral population of ~11,750 individuals experiencing a bottleneck to ~3,300 individuals ~5,500 years ago, followed by a further exponential decline beginning ~35 years ago resulting in a current population size of ~1,300 individuals (Terbot et al. 2025). Simulations using SLiM were run forward-in-time, which additionally allowed for the use of a DFE for exonic regions to account for expected purifying and background selection effects (following Soni et al. 2025b; and see Johri et al. 2023; Soni and Jensen 2025). As prior work found the mutation rate in males to be ~2.7 times greater than that of females (Versoza et al. 2025a), along with the difference in time spent in males relative to females for the X chromosome, we calculated an average rescaled rate of 2.22×10^{-8} mutations per site per generation for males, and 0.82×10^{-8} mutations per site per generation for females. While another recent study (Wang et al. 2025) suggested the female mutation rate to exceed that of males, the generalization of those results is highly questionable in any long-term evolutionary context, given 1) that their pedigree data set was biased by old age female aye-ayes (and there exists a strong age bias in mutation rates; Versoza et al. 2025a), 2) the bias was not confirmed in that study when evaluated in a larger phylogenetic context, and, 3) the finding would be unprecedented compared to almost every other study of male versus female mutation rates in mammals conducted to date (see e.g., Bergeron et al. 2023). Mutations were modeled for the entire non-PAR of the X chromosome as being strictly neutral in non-exonic regions, and drawn from a DFE in exonic regions. Sex ratios for the simulated populations were set to one of three different levels of male:total ratios (0.25, 0.5, and 0.75). From these simulations, variant call files (in. vcf format) of five unrelated samples (three females and two males) were produced to mimic the empirical data. Repetitive and constrained regions of these files were then masked to match empirical masking, and SFS were produced. From these simulated masked variant call files, we calculated the mean SFS and compared it with that obtained from the empirical data.

2.5 | Inferring Fine-Scale Recombination and Mutation Rate Maps of the X Chromosome

Following the approach outlined in Soni et al. (2025c), we used the demography-aware estimator pyrho v.0.1.7 (Spence and Song 2019) to infer fine-scale recombination rates across the X chromosome and converted the population-scaled recombination rate (ρ) to the per-generation recombination rate (r). After visual inspection of the cumulative map, we removed a 192 kb-long region (88,682,220 – 88,874,241 bp) which showed a ~40-fold excess in recombination compared to the median rate in the non-PAR and which overlapped with a region of excessive read coverage, thus likely resulting from an assembly error. In addition to these indirect estimates, we applied the methodology described in Versoza et al. 2025c) to directly identify female crossover events from the pedigree and then rescaled the indirect estimates such that the genetic map length inferred by pyrho is equal to that observed in the pedigree, thereby accounting for the lack in recombination in males.

Following the approach of Soni et al. (2025c) for mutation rate inference, we replaced the previously existing low-coverage aye-aye X chromosome from the Kuderna et al. (2024) 223-way primate multiple species alignment with the high-quality, annotated aye-aye X chromosome of Versoza and Pfeifer (2024) using the Cactus pipeline (Armstrong et al. 2020). Using this updated multiple-species alignment, substitutions along the aye-aye branch were extracted in both neutral and functional regions, removing any divergent sites observed to be polymorphic. Neutral divergence rates were calculated by considering the number of divergent sites relative to accessible sites in a given window, and exonic divergence by considering the number of divergent sites relative to exonic length. Rates were calculated in windows of 1 kb, 10 kb, 100 kb, and 1 Mb, with step sizes equal to half the window size. To convert neutral divergence into estimated per-generation mutation rates, a divergence time of 54.9 million years (Horvath et al. 2008; Soni et al. 2025b) was divided by a generation time of 5 years (Ross 2003; Louis et al. 2020).

2.6 | Scans for Positive and Balancing Selection on the X Chromosome

To investigate the potential of recent, rare bouts of positive or balancing selection, we used the demographic model described above and the previously mentioned simulations under that model to determine statistical thresholds for performing genomic scans. To evaluate evidence for recent selective sweeps, we used

SweepFinder2 v.1.0 (Nielsen et al. 2005; DeGiorgio et al. 2016) on each SNP in the 100 simulated X chromosomes (*-su*). From this, we established a maximum Composite Likelihood Ratio (CLR) for the aye-aye X chromosome under the estimated population history, accounting for purifying and background selection effects in coding regions. We similarly evaluated evidence for recent balancing selection, utilizing BalLerMix v.2.5 (Cheng and DeGiorgio 2020) on all simulated X chromosomes using the $B_{0,}$

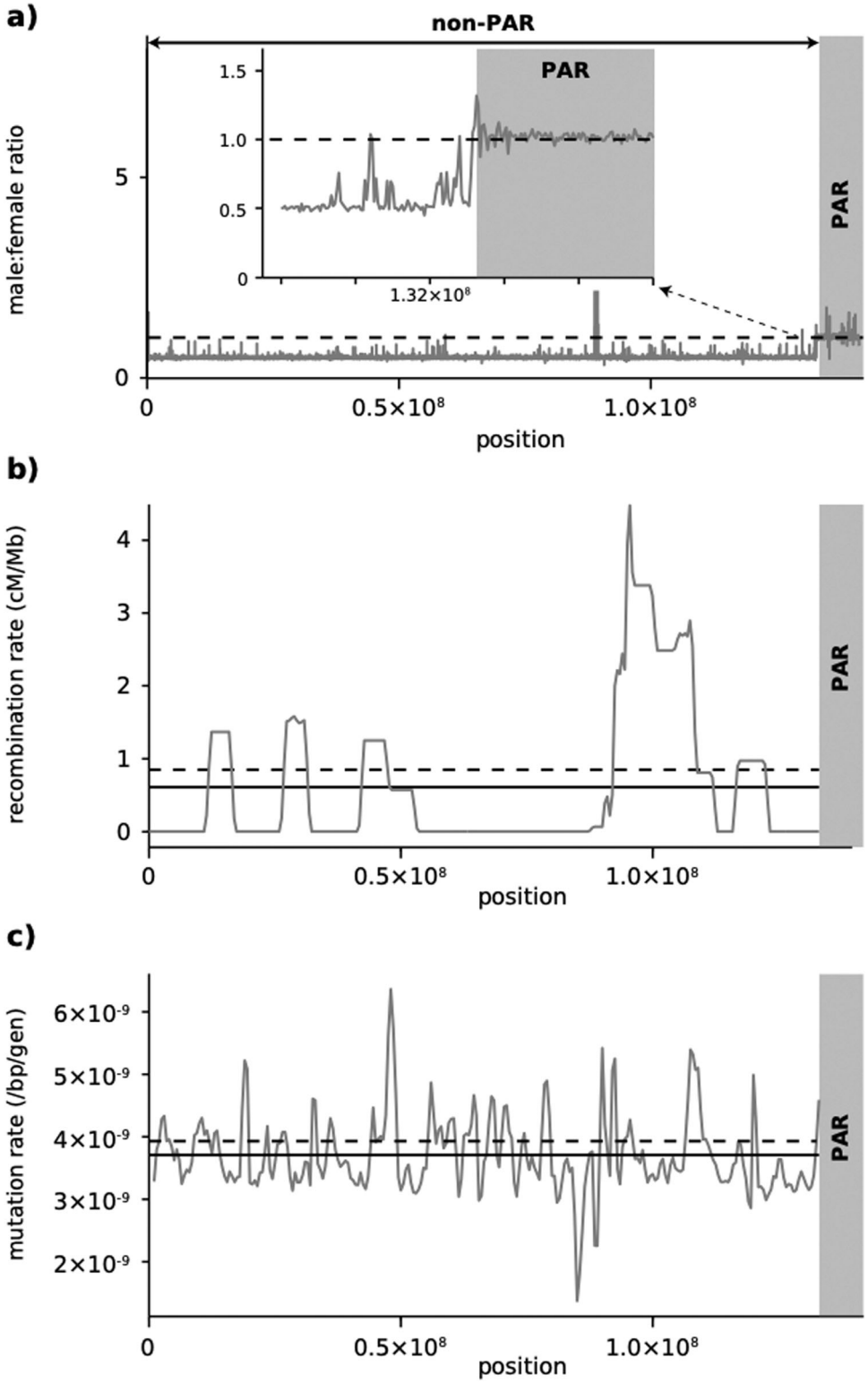

FIGURE 1 | (a) Male:female sequence coverage ratios on the X chromosome were used to identify the pseudoautosomal boundary, separating the pseudoautosomal region (PAR) from the completely sex-linked non-PAR. In the former, a male:female coverage ratio of 1 (indicated by a dashed line) is expected as males and females carry the same number of copies of that region. (b) Recombination and (c) mutation rate maps across the non-PAR. The solid line on each panel indicates the average rates for the X chromosome; the dashed line on each panel indicates the genome-wide average rates for the autosomal chromosomes (Soni et al. 2025c; Versoza et al. 2025a,c).

$_{MAF}$ method – which uses the folded SFS – with 10 and 100 SNP windows and step sizes of 5 and 50, respectively (*-w 10,--step 5* and *-w 100,--step 50*).

3 | Results & Discussion

3.1 | Determining the Location of the Pseudoautosomal Boundary

To determine the location of the completely sex-linked segment of the X chromosome – the focus of this study – we used differences in male:female coverage to identify the pseudoautosomal boundary separating the non-PAR from the PAR. Notably, earlier work was unable to reliably identify the size of the PAR in aye-ayes (Shearn et al. 2020), owing to the noisiness of medium coverage data from a single representative of each sex mapped to the highly-fragmented genome of the distantly-related gray mouse lemur (*Microcebus murinus*, Mmur_2.0 consisting of > 10,000 scaffolds). However, the recently released, highly-contiguous genome assembly for the species allowed us to confidently identify this region (Figure 1a).

3.2 | Estimating the Demographic History of the X Chromosome

With the pseudoautosomal boundary at hand, we first characterized the population structuring in the non-PAR. The first lowest cross-validation error for the analyses using ADMIXTURE (Alexander et al. 2009) was for a single deme (cross-validation errors for $K = 1$, 1.56609; $K = 2$, 2.01019; $K = 3$, 1.85951; $K = 4$, 0.43792; $K = 5$, 0.00002); all cross-validation errors lower than the single deme's value occurred after an increase in cross-validation errors for two source demes. This is consistent with the 'non-North', autosomal-based assignments of Terbot et al. (2025). Simulating their demographic model (see Methods), re-scaled for the X chromosome, led to a strong fit to the empirically observed SFS (Figure 2). These results thus suggest that an alternative X chromosome-specific demographic history is not needed to fit the observed data. Based upon this demographic history, we next inferred the likely sex ratios characterizing the long-term evolutionary dynamics of this population. As shown in Figure 2, a 0.5 male:total ratio was found to be most consistent with the data (despite previously described ecological differences in territorial ranges between the sexes), with alternative sex ratios generally reducing variation below the empirical observation and skewing the SFS away from that observed. These relative differences between alternative sex ratios are consistent with expectations as have been described via simulation (e.g., Spurley and Payseur 2025).

3.3 | Inferring Fine-Scale Recombination and Mutation Rate Maps of the X Chromosome

Estimation of the female genetic map length based on the crossovers observed in the three-generation pedigree yielded a total length of 120 cM in the non-PAR – comparable to previously obtained female genetic map length estimates for chromosomes 8 (~ 110 cM) and 10 (~ 120 cM) in aye-ayes (Versoza et al. 2025c) which are of similar physical length (chromosome 8: 163 Mb; chromosome X: 132 Mb; chromosome 10: 115 Mb; Versoza and Pfeifer 2024) but shorter than the length inferred in humans (~ 140 cM, though note that the non-PAR is longer in humans than in aye-ayes; Kong et al. 2002). The average female recombination rate in the non-PAR was 0.91 cM/Mb, slightly lower than both the average female recombination rate in the aye-aye autosomes (~ 0.94 cM/Mb; Versoza et al. 2025c) and the human non-PAR (~ 0.94 cM/Mb; Kong et al. 2002). Under the even sex ratios inferred, chromosome X spends 2/3rd time in

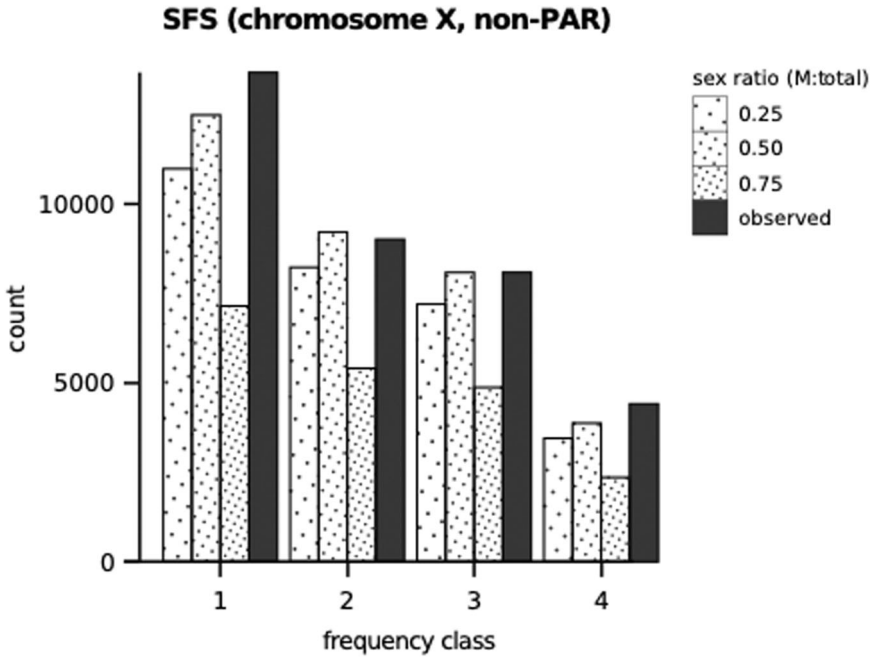

FIGURE 2 | Site frequency spectra (SFS) of neutral sites in the non-pseudoautosomal region (non-PAR) of the X chromosome for the observed data (filled), and average values for simulated data at different sex ratios (0.25 male (M) vs male+female (total), sparsely dotted; 0.5 M:total, moderately dotted; 0.75 M:total, heavily dotted).

female aye-ayes. Thus, due to the absence of recombination whilst in males, amongst other differences (Charlesworth 2017; Olito and Abbott 2023), the sex-averaged recombination rate on the X is 0.61 cM/Mb (Figure 1b), approximately 30% lower than the sex-averaged rate previously reported for the autosomes (0.85 cM/Mb; Versoza et al. 2025c), as expected. In contrast, the neutral mutation rate inferred across the X chromosome was found to be consistent with both directly and indirectly inferred autosomal rates, suggesting a mean rate of 3.78 $\times 10^{-9}$ mutations per base pair per generation (Figure 1c). This overall mutation rate is low relative to other primates (see Bergeron et al. 2023 and references therein), which may correspond to hypothesized reduced rates of speciation and diversification on the strepsirrhine branch (Scott 2022).

3.4 | Scans for Positive and Balancing Selection on the X Chromosome

Using a well-fit evolutionary baseline model based on the inferred demographic history of the population and estimated mutation and recombination rates, we additionally constructed thresholds for performing scans of selection. As expected, we found extensive evidence for purifying selection, and thus included these resulting selective effects in and around exons via a modeling of the DFE (see Methods). The maximum observed CLR value for the simulated chromosomes using SweepFinder2 (Nielsen et al. 2005; DeGiorgio et al. 2016) – designed to detect recent selective sweeps – was 217.60. In comparison, the maximum empirical CLR observed (130.11) was considerably below this threshold (Figure 3a). The maximum CLR for the simulated data using BalLerMix (Cheng and DeGiorgio 2020) – designed to detect balancing selection – was 19.54 for 10 SNP windows and 154.98 for 100 SNP windows. By comparison, the maximum CLR values for the observed data was 16.86 for the 10 SNP windows and 47.12 for the 100 SNP windows (Figure 3b,c). Thus, the maximum baseline model CLR values were well above the maximum observed CLR values.

As such, we found no significant evidence for either episodic positive or balancing selection in the observed data once

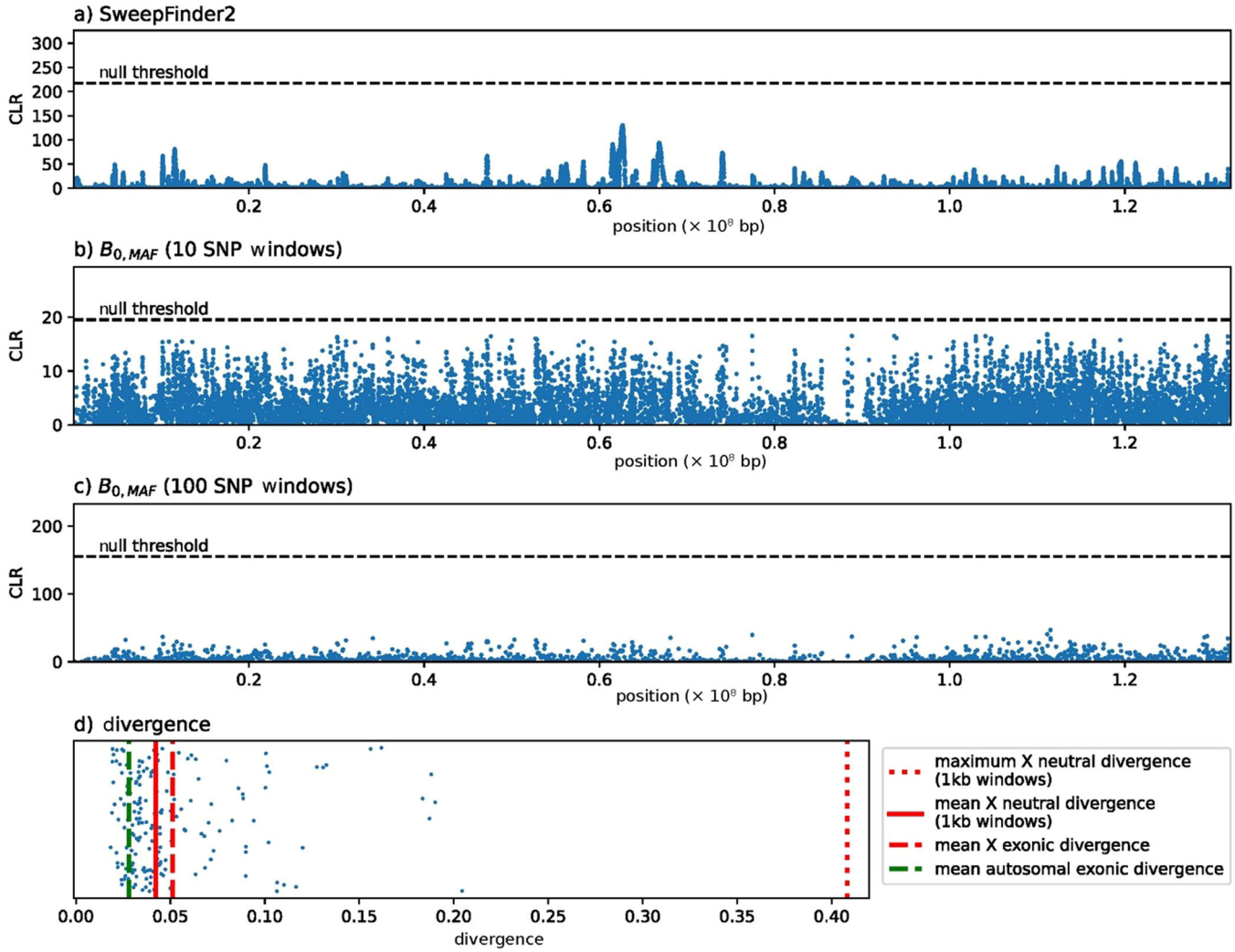

FIGURE 3 | Polymorphism- and divergence-based scans for selection on the X chromosome using (a) SweepFinder2, (b), BalLerMix in 10 SNP windows, and (c) BalLerMix in 100 SNP windows, in which null thresholds (dashed lines) for composite likelihood ratios (CLR) were determined via simulation. (d) Divergence across the X chromosome, with each point representing an X-linked exon. The mean exonic divergence on the X (dashed red line), the mean and maximum neutral divergence on the X in 1 kb windows (solid and dotted red lines, respectively), and the mean autosomal exonic divergence (dashed green line) are provided for comparison.

TABLE 1 | Mean, standard deviation (std.), and maximum (max) neutral divergence on chromosome X based on window size, for windows containing at least 200 accessible sites. The equivalent values from the autosomes are provided for comparison (Soni et al. 2025c).

	Autosomal divergence			X Divergence		
Window size	**Mean**	**Std.**	**Max**	**Mean**	**Std.**	**Max**
1 kb	0.0437	0.0263	0.4941	0.0424	0.0288	0.4082
10 kb	0.0439	0.0165	0.2681	0.0412	0.0235	0.3364
100 kb	0.0432	0.0088	0.1352	0.0415	0.0121	0.1415
1 Mb	0.0432	0.0059	0.0730	0.0418	0.0080	0.0734

accounting for the evolutionary processes certain-to-be-occurring via the construction of an appropriate evolutionary baseline model. Overall, this suggests that, if recent sweeps or periods of balancing selection have occurred on the X chromosome, they are not identifiable within the context of this baseline model (see Poh et al. 2014; Johri et al. 2022; Jensen 2023). Moreover, no exons were observed to be diverging more rapidly than the most rapid neutral observation (Figure 3d), once accounting for windows of like size (Table 1). The lack of accelerated divergence in any exonic region of the X chromosome further indicates a lack of recurrent, long-term adaptive processes on this chromosome. Taken together with the polymorphism-based scans, these results therefore suggest limited evidence for either strong and recent, or rapidly recurrent, adaptive evolution across X-linked genes in the aye-aye genome. This is in contrast to recent autosomal analyses, which found strong support for long-term balancing selection acting on olfactory-related genes in this species (Soni et al. 2025a).

4 | Concluding Thoughts

In summary, our results provide unique insights into the X chromosome dynamics of a strepsirrhine, including the first fine-scale demarcation of the PAR in aye-ayes, a quantification of male:female sex ratios otherwise extremely difficult to obtain in this elusive, nocturnal, endangered species, a characterization of the fine-scale recombination landscape, and further support for generally reduced mutation rates in lemurs relative to other primates. We did not find any evidence for positive or balancing selection on the X chromosome over either recent or deep history, which may be interpreted to fit with the general finding of a 'slow-X evolution' observed in other primates (e.g., Xu et al. 2011). As a highly endangered species (IUCN 2024), understanding the demographic history and evolutionary forces driving the aye-aye's evolution, including those that may be unique to their sex chromosomes, is critical for effective population management (Orkin et al. 2021). While we did not find any evidence for a biased sex ratio, it is important to emphasize that conservation of all reproductively active adults is essential for population survival, and the prolonged inter-birth interval of aye-ayes may only serve to further increase the long-term vulnerability of the species (Brook et al. 2018).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

This project was based on sequence data available under NCBI BioProjects PRJNA1085541, PRJNA1179987, and PRJNA1181251. All scripts are available at the GitHub repository: https://github.com/jwterbot2/ayeaye_Xchromosome.

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