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RESEARCH ARTICLE

Estimating the Distribution of Fitness Effects in Aye-Ayes (*Daubentonia madagascariensis*), Accounting for Population History as Well as Mutation and Recombination Rate Heterogeneity

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ABSTRACT

The distribution of fitness effects (DFE) characterizes the range of selection coefficients from which new mutations are sampled, and thus holds a fundamentally important role in evolutionary genomics. To date, DFE inference in primates has been largely restricted to haplorrhines, with limited data availability leaving the other suborder of primates, strepsirrhines, largely underexplored. To advance our understanding of the population genetics of this important taxonomic group, we here map exonic divergence in aye-ayes (*Daubentonia madagascariensis*)—the only extant member of the Daubentoniidae family of the Strepsirrhini suborder. We further infer the DFE in this highly-endangered species, utilizing a recently published high-quality annotated reference genome, a well-supported model of demographic history, as well as both direct and indirect estimates of underlying mutation and recombination rates. The inferred distribution is generally characterized by a greater proportion of deleterious mutations relative to humans, providing evidence of a larger long-term effective population size. In addition however, both immune-related and sensory-related genes were found to be amongst the most rapidly evolving in the aye-aye genome.

1 | Introduction

The distribution of fitness effects (DFE) summarizes the range of selection coefficients from which new mutations are sampled. Consequently, characterizing the DFE holds a fundamentally important role in evolutionary genomics, as it quantifies the fraction of neutrally evolving genomic mutations, provides insights into the expected relative frequencies of purifying relative to positive selection, and informs the expected effects of selection at linked sites, to name but a few implications (see the reviews of Eyre-Walker and Keightley 2007; Keightley and Eyre-Walker 2010; Bank et al. 2014a). Moreover, given that the vast majority of fitness-impacting mutations are deleterious, the constant elimination of these variants via purifying selection and the associated background selection (BGS) effects (Charlesworth et al. 1993) represent constantly

Susanne P. Pfeifer and Jeffrey D. Jensen are co-corresponding authors and jointly supervised the project.

Summary

- The distribution of selective effects that characterizes newly arising mutations is fundamental for understanding evolutionary outcomes, and we here infer this distribution in a highly-endangered primate, the aye-aye (*Daubentonia madagascariensis*).
- While the general shape of the distribution suggests stronger purifying selection effects relative to humans, we have additionally identified faster-evolving functional categories which include both immune-related and sensory-related genes.
- As aye-ayes are gravely threatened owing to ongoing deforestation in Madagascar, this study will help to better understand the long-term selective dynamics of the species, the only extant member of the Daubentoniidae family.

operating processes shaping levels and patterns of genomic variation in and around functional regions. As such, an accurate characterization of these effects is critical for the construction of any evolutionary baseline model for a given species (Comeron 2014, 2017; Poh et al. 2014; Irwin et al. 2016; Johri et al. 2022a; Howell et al. 2023; Terbot et al. 2023; Soni et al. 2023; Soni and Jensen 2025), and, because these effects may differ strongly depending on the relative proportion of weakly relative to strongly deleterious mutations, the DFE shape again emerges as a fundamental component for any evolutionary modeling or inference (Charlesworth et al. 1993; Hudson and Kaplan 1994; Charlesworth et al. 1995; Ewing and Jensen 2014, 2016; Johri et al. 2020).

Generally speaking, there are two classes of DFE inference, one applicable to lab-tractable organisms that may be experimentally evolved, and one applicable to natural population analysis. The former includes mutation accumulation experiments—in which a population of organisms can be maintained often in replicate, sampled at regular intervals, and the fitness effects of newly arising mutations characterized with respect to, for example, the wildtype state (e.g., Lenski et al. 1991; Barrick and Lenski 2013; Desai 2013; Böndel et al. 2019; Morales-Arce et al. 2022; Crombie et al. 2024). This class also includes mutagenesis experiments—in which hundreds or thousands of individuals can be maintained that carry one or very few mutations, and their fitness assessed by, for example, relative growth rates (e.g., Hietpas et al. 2011; Jacquier et al. 2013; Bank et al. 2014b; Fowler and Fields 2014; Matuszewski et al. 2015). Both methods represent powerful DFE inference approaches for the organisms in which they can be applied (e.g., *Saccharomyces cerevisiae*, *Caenorhabditis elegans, Chlamydomonas reinhardtii*), with the caveat being that they provide DFE inference only within the context of a lab-grown environment.

With regard to natural population analysis, which will be our focus here, there are generally approaches utilizing divergence data, polymorphism data, or a combination of both. Perhaps the most basic approach utilized to infer aspects of the DFE relies on comparisons between nonsynonymous and synonymous divergence. Assuming that synonymous sites are effectively neutral, and thus characterized by a substitution rate equal to their mutation rate (Kimura 1968), one may quantify the fraction of nonsynonymous mutations that are deleterious (and thus characterized by reduced fixation probabilities relative to neutrality) by assessing the depression in non-synonymous divergence relative to the synonymous neutral standard (e.g., Eyre-Walker et al. 2002). Similarly, if advantageous mutations are present (characterized by increased fixation probabilities relative to neutrality), one may assess this fraction of the DFE via the acceleration of non-synonymous divergence relative to synonymous (e.g., Smith and Eyre-Walker 2002). These advances largely owed to the realization that a McDonald-Kreitman-style test (McDonald and Kreitman 1991) could be used to infer proportions of adaptive substitutions (Charlesworth 1994). Synonymous and non-synonymous mutations aside, one may similarly utilize this divergence-based logic to assess selective constraints acting in different genomic regions (e.g., coding relative to intronic relative to intergenic; Andolfatto 2005).

When incorporating polymorphism data into DFE inference, one initial challenge is the need to incorporate the demographic history of the population into the inference procedure, given that this history may also act to shape levels and patterns of variation and thus may potentially result in mis-inference if unaccounted for (see the review of Johri et al. 2022b). One of the first advances in this regard utilized the site frequency spectrum (SFS) at putatively neutral synonymous or noncoding sites to infer a population history, and then conditioned on that history to infer the DFE at putatively functional non-synonymous sites (Williamson et al. 2005; Keightley and Eyre-Walker 2007). Such step-wise approaches yielded some of the first polymorphism-based DFE estimates for a variety of organisms (Eyre-Walker and Keightley 2007, 2009; Boyko et al. 2008; Schneider et al. 2011). A related category of methods also arose for utilizing time-sampled polymorphism data to infer individual mutational effects based on observed allele frequency changes—as may be applicable to ecological datasets or ancient DNA sampling—with the stochastic effects of genetic drift associated with the given population history being incorporated by estimating an effective population size based on the variance observed in neutral allele frequencies (e.g., Malaspinas et al. 2012; Foll et al. 2015; Ferrer-Admetlla et al. 2016; and see the review of Malaspinas 2016). However, in addition to generally being limited to relatively simple population-size change models (though more complex models have been developed; e.g., Ma et al. 2023; Kim et al. 2017), these initial single- and multi-timepoint approaches also assumed independence amongst sites, and thus neglected any role of background selection or other forms of genetic hitchhiking in further shaping levels of polymorphism (see the reviews of Charlesworth and Jensen 2021, 2022).

To address these polymorphism-based challenges, simultaneous inference approaches have recently been developed. Though accounting for the effects of selection on linked sites within an analytical framework remains challenging, Cvijović et al. (2018) obtained expressions for the SFS at sites experiencing BGS in a constant size population, and Friedlander and Steinrücken (2022) described a numerical framework to obtain expected SFS and linkage disequilibrium (LD) patterns around a selected region with changing population size. To allow for more

complex models, progress has also been made using approximate Bayesian computation (ABC) approaches with forward simulations, to model both complex population histories and flexible DFE shapes, whilst accounting for the resulting effects of selection on linked sites. For example, Johri et al. (2020) developed a joint ABC approach estimating the DFE densities of neutral, weakly deleterious, moderately deleterious, and strongly deleterious mutations, together with a history of population size change, utilizing aspects of the SFS, LD, and divergence as summary statistics. Notably, the exclusion of BGS effects in previous methods was found to result in an underestimation of weakly deleterious mutations and an overestimation of population growth – a bias that is corrected within this ABC framework (Johri et al. 2021). Subsequent work has also demonstrated the potential mis-inference that may arise by neglecting underlying heterogeneity in rates of both mutation and recombination (Soni et al. 2024). Taken together, this literature thus emphasizes the importance of incorporating population history, the effects of selection at linked sites, as well as mutation and recombination rate maps/uncertainties when performing DFE inference.

In primates specifically, these various divergence- and polymorphism-based approaches have been employed widely, with humans being the best studied in this regard. For example, Keightley and Eyre-Walker (2007) fit a gamma-distributed DFE utilizing a gene set associated with severe disease or inflammatory response, and estimated a large proportion (~40%) of strongly deleterious mutations and a relatively low proportion (~20%) of effectively neutral mutations. Huber et al. (2017) utilized a wider selection of genes, resulting in a DFE skewed towards effectively neutral mutations (~50%; similar to the estimate of Johri et al. 2023 utilizing a different subset of genes), and a smaller proportion (~20%) of strongly deleterious mutations. Thus, these differences may well simply and accurately reflect true DFE differences in the underlying gene sets evaluated. Similar inference has also been performed across the great apes (e.g., Castellano et al. 2019; Tataru and Bataillon 2020), and considerations have been extended to general regulatory regions as well (e.g., Simkin et al. 2014; Anderson et al. 2020; Kuderna et al. 2024).

Notably however, owing largely to data availability, these estimates have been performed primarily in haplorrhines (specifically in the great apes), with the other suborder of primates, strepsirrhines, being largely unexplored. Yet, a number of recent advances have uniquely enabled investigation in this neglected space of the primate clade. Firstly, Versoza and Pfeifer (2024) have recently provided an annotated chromosome-level genome assembly for aye-ayes (*Daubentonia madagascariensis*)—the only extant member of the Daubentoniidae family of the Strepsirrhini suborder—thereby allowing for the essential demarcation of functional and non-function genomic regions needed for performing DFE inference. Secondly, recent work has also generated high-quality direct mutation and recombination rate estimates for aye-ayes from multi-generational pedigree data (Versoza et al. 2024, 2025; Versoza, Lloret-Villas, et al. 2025)—as well as indirect fine-scale estimates based on autosomal patterns of LD and neutral divergence (Soni, Versoza, et al. 2024). Finally, utilizing high-coverage whole-genome data from unrelated individuals, Terbot et al. (2025) recently estimated a well-fitting population history for aye-ayes (and see Soni et al. 2025)—which described a severe and ancient population size decline likely associated with the human colonization of Madagascar, as well as a more recent decline likely associated with habitat destruction—thereby providing the needed accounting of the role of population history in shaping observed SFS across the genome. Based on these advances, we here quantify exonic divergence and infer the DFE characterizing this species.

2 | Materials and 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 Principles for the Ethical Treatment of Nonhuman Primates.

2.2 | Exonic Divergence

To obtain exonic divergence values, we first utilized the *halRemoveGenome* function implemented in HAL v.2.2 (Hickey et al. 2013) to remove the outdated aye-aye genome assembly from the 447-way multiple species alignment (which consists of the combined mammalian multiple species alignment of Genereux et al. (2020) and the primate multiple species alignment of Kuderna et al. [2024]). We then extracted the ancestral genomes PrimatesAnc005 and PrimatesAnc011 from the 447-way alignment using HAL's *hal2fasta* function, and aligned them with the current NCBI reference genome (DMad_hybrid; GenBank accession number: GCA_044048945.1; Versoza and Pfeifer 2024) in Cactus v.2.9.2 (Armstrong et al. 2020), maintaining the branch lengths previously inferred in the 447-way alignment. Notably, although the previous version of the aye-aye assembly included in the 447-way multiple species alignment (ASM2378347v1 generated from single-molecule long-read PacBio data; Shao et al. 2023) was both of high-quality and near-complete (containing 98.82% of single-copy orthologous genes that are highly-conserved amongst eukaryotes at the genome level), the lack of detailed gene annotations previously prevented the application of divergence- and polymorphism-based approaches to infer the DFE. At the same time, the strong similarity between this earlier assembly and the more recent, fully annotated aye-aye assembly of Versoza and Pfeifer (2024) allowed us to take advantage of the alignment previously generated by Kuderna et al. (2024). As a final alignment step, we used HAL's *halReplaceGenome* function to attach the new sub-alignment back into the 447-way alignment.

To infer exonic divergence, we retrieved 'point mutations' between the aye-aye and PrimateAnc005 via HAL's *halSummarizeMutations*

function. This gave us all substitutions along the aye-aye branch (i.e., substitutions between the aye-aye and PrimateAnc005). Notably, this previously reconstructed ancestor is of high-quality as the previous version of the aye-aye assembly was sufficient for divergence-based inference; however, the recent genome annotations of Versoza and Pfeifer (2024) provided the critical component of being able to differentiate functional from nonfunctional genomic regions. Additionally, the inclusion of high-quality polymorphism data (Soni, Versoza et al. 2024; Soni et al. 2025; Terbot et al. 2025) allowed for the demarcation between truly divergent sites and sites that are segregating in the population. Finally, we masked all point mutations that were not located in exons, and calculated exonic divergence by dividing the number of divergent sites in each exon by the total accessible exonic length. Notably, the underlying variance in divergence is expected to scale with gene length; however, we sub-setted our exons to include only those with a minimum of 100 bp of accessible sites, and all calculated comparisons were at the per-site level. These calculations thus represent total exonic divergence (i.e., including both synonymous and non-synonymous fixations within the coding boundaries).

2.3 | Gene Functional Analysis

Utilizing the aye-aye genome annotations of Versoza and Pfeifer (2024), we calculated mean divergence per site per gene, and performed gene functional analysis using g:Profiler (Kolberg et al. 2023) on the subset of genes with a divergence value greater than the 75th percentile of neutral divergence in aye-ayes (0.0397; Soni, Versoza et al. 2024). The Supporting File provides detailed information on the gene functional analysis, the corresponding *p*-values of each functional category, the gene sets belonging to each category, as well as the observed gene-level divergence.

2.4 | DFE Inference

To fit a DFE to our exonic divergence, we simulated an exonic region of length 2978 bp (i.e., the mean empirical length of exons of size greater than 1 kb; see Supporting Figure S1 for the observed exonic length distribution) in SLiM v.4.0.1 (Haller and Messer 2023) under the Terbot et al. (2025) aye-aye demographic model, assuming 54.9 million years since the branch split (Horvath et al. 2008) and a generation time of 5 years (Ross 2003; Louis et al. 2020) for 100 replicates. While directly matching empirical exonic lengths is important when performing DFE inference based on polymorphism data owing to differing expected background selection effects between larger and smaller functional regions (see Johri et al. 2020, 2021), as we here performed inference based on per-site, per-gene divergence data, this simulation framework is appropriate given that background selection effects do not modify neutral divergence rates (Birky and Walsh 1988). Simulations included a $10N_{\text{ancestral}}$ generation burn-in time before the demographic model (where $N_{\text{ancestral}}$ is the initial population size). Each simulation replicate had mutation and recombination rates drawn from a normal distribution, such that the mean rates across all 100 simulation replicates were equal to the mean pedigree-estimated rates of 0.4e-8/bp/generation and 0.85 cM/Mb for mutation and recombination, respectively (Versoza et al. 2025; Versoza, Lloret-Villas et al. 2025). Following Johri et al. (2020), exonic mutations were drawn from a DFE comprised of fixed classes, denoted by $2N_{\text{ancestral}}\ s < 10$ (i.e., nearly neutral mutations), $10 \leq 2N_{\text{ancestral}}\ s < 100$ (i.e., weakly/moderately deleterious mutations), and $100 \leq 2N_{\text{ancestral}}\ s$ (i.e., strongly deleterious mutations), where s is the reduction in fitness of the mutant homozygote relative to wildtype. Notably, this DFE represents that of all newly arising mutations (e.g., although strongly deleterious mutations would neither be expected to segregate in the population nor reach fixation with any appreciable probability, their removal via purifying selection is nonetheless an important component of the underlying evolutionary model). One may alternatively consider the DFE of those mutations reaching sufficient frequency in the population to be sampled as segregating variants, which is by definition a subset of the DFE of new mutations. One may also consider the DFE of mutations reaching fixation in the population, which itself in turn is necessarily a subset of the DFE of segregating variants (see the reviews of Eyre-Walker and Keightley 2007; Bank et al. 2014a).

We performed a grid search to infer the DFE parameters in our aye-aye population, which were the proportions of mutations drawn from each DFE category. Using the DFE inferred by Johri et al. (2023) in humans as a starting point, we simulated 100 replicates for each parameter combination, and compared the fit of exonic divergence between our empirical and simulated data. For the simulated data, exonic divergence was calculated as the number of fixations postburn-in (i.e., across the aye-aye divergence time), allowing us to directly compare the empirically observed number of divergent sites along the aye-aye branch with the number of fixations accrued in our simulated population during the divergence phase. Resulting values from the grid search were visually compared with the empirical data to assess fit.

3 | Results and Discussion

3.1 | Interpreting Exonic Divergence

Building upon recent advances in aye-aye genomics, we replaced the original aye-aye genome in the 447-way mammalian multiple species alignment (Genereux et al. 2020) that includes hundreds of closely related primate species (Kuderna et al. 2024) with the high-quality aye-aye reference genome of Versoza and Pfeifer (2024) to quantify fine-scale exonic divergence in the species. Figure 1 summarizes observed exonic divergence on the aye-aye branch, with the maximum neutral divergence for 1 kb and 1 Mb windows calculated from nonfunctional genomic regions (see Soni, Versoza et al. 2024) provided for orientation. Although a considerable number of exons were characterized by rates of fixation greater than the maximum neutral divergence observed in 1 Mb windows, no exons were found to be in excess of the neutral rate observed in 1 kb windows—the more appropriate comparison given the mean exonic length. Thus, exonic divergence was observed to be lower than the maximum neutral divergence in aye-ayes without exception, as expected from the dominant action of purifying selection in functional regions (Charlesworth et al. 1993).

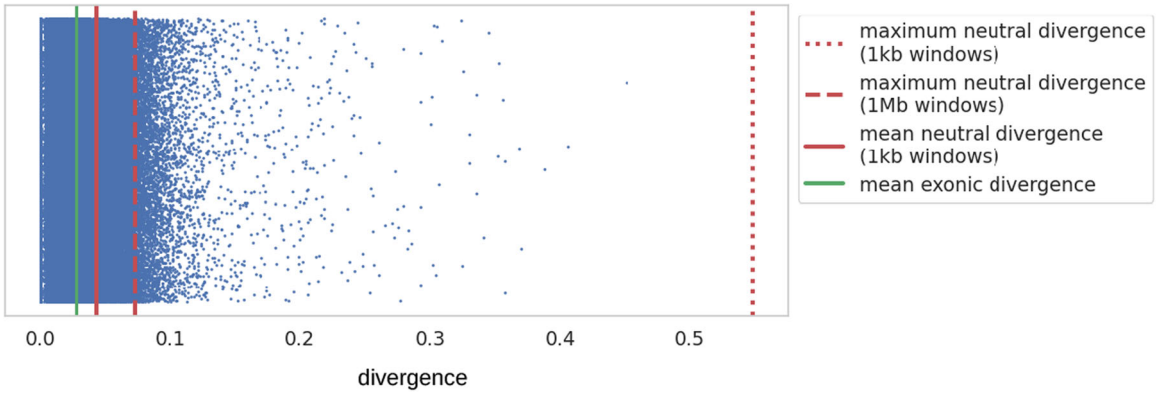

FIGURE 1 | Exonic divergence scatter plot with maximum neutral divergence values marked for windows of size 1 Mb (red dashed line) and 1 kb (red dotted line), as well as the mean neutral divergence for 1 kb windows (red solid line), as calculated from nonfunctional regions of the aye-aye genome (see Soni, Versoza, et al. 2024). Each dot represents an autosomal exon, and the mean exonic divergence as calculated in this study is plotted (green solid line).

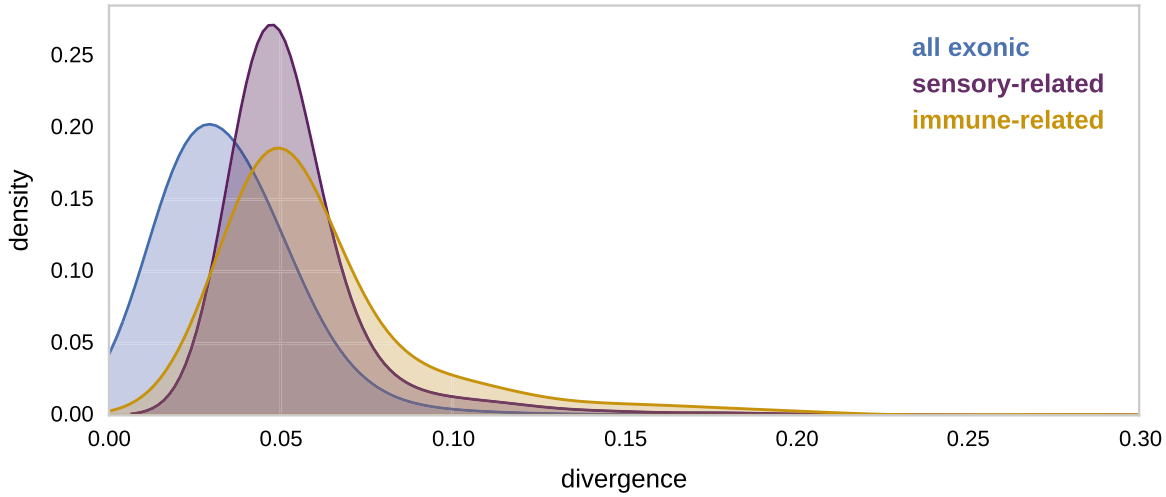

FIGURE 2 | Density plots of exonic divergence in aye-ayes for all exons (blue), exons located in genes implicated in sensory-related functions (purple), and exons located in genes implicated in immune-related functions (gold).

Furthermore, given this observation, the possibility that some or all of these accelerated subsets of genes may instead be experiencing relaxed selective constraint cannot be ruled out.

However, given that even recurrent positive selection is expected to be rare relative to purifying selection, the absence of entire exons evolving faster than neutrality neither itself eliminates the possibility of positive selection contributing to exonic divergence in these accelerated subsets of genes. For example, distinct classes of exons were found to occupy the tails of the exonic divergence distribution, and were found to be in excess of the mean neutral fixation rate. Utilizing the genome annotations from the Versoza and Pfeifer (2024) reference genome to calculate the mean divergence per gene, we ran a gene functional analysis using g:Profiler (Kolberg et al. 2023) on all coding regions with a mean divergence greater than the 75th percentile of neutral divergence (0.0397). Figure 2 provides the divergence distribution of all examined exons, compared with the distributions of the two fastest-evolving gene classes—those related to sensory and immune function.

Immune-related genes have long been observed to be amongst the most rapidly evolving across vertebrates, as populations continually respond to challenges of pathogen exposure (e.g., George et al. 2011; Rausell and Telenti 2014), and our results remain consistent with this pattern. With regard to the sensory-related distribution, both the nocturnal activity patterns of aye-ayes (with the suggestion previously being made that dichromacy may enable aye-ayes to perceive color whilst foraging in moonlight conditions; Perry et al. 2007), together with evidence that aye-ayes may discriminate between individuals based on scent (Price and Feistner 1994) and use scent-marking to attract mates (Winn 1994), both suggest potentially significant roles for opsin- and olfactory-related genes throughout the evolutionary history of the species. Relatedly, Soni et al. (2025) recently found that a number of sensory functional categories including G-protein coupled receptors and olfactory receptors had strong statistical support for being maintained by long-term balancing selection in aye-ayes – noting that diversity in these genes may increase the number of different odorant-binding sites (Lancet 1994) – further supporting these hypotheses.

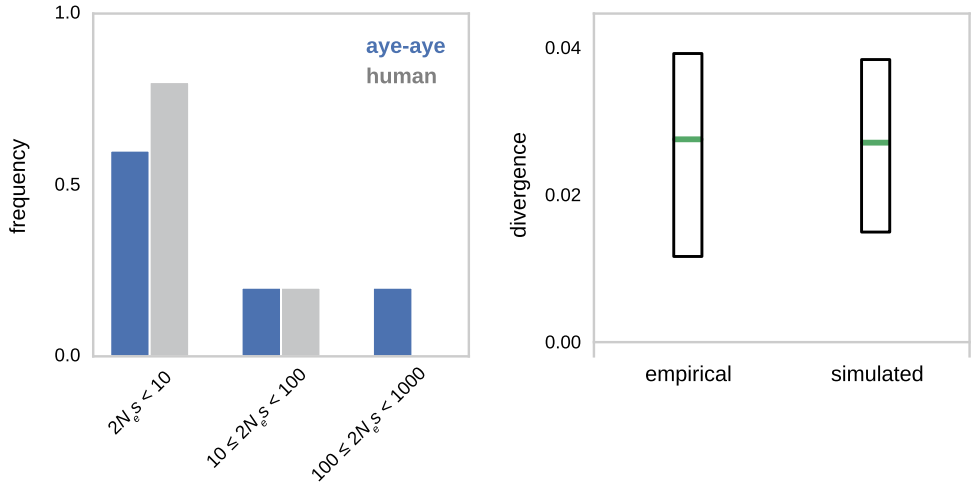

FIGURE 3 | Comparison of the empirical and simulated divergence under the best-fitting DFE. Exonic mutations were drawn from a DFE comprised of fixed classes, denoted by $2N_{\text{ancestral}}\ s < 10$ (i.e., nearly neutral mutations), $10 \leq 2N_{\text{ancestral}}\ s < 100$ (i.e., weakly/moderately deleterious mutations), and $100 \leq 2N_{\text{ancestral}}\ s$ (i.e., strongly deleterious mutations). Left panel: Best-fitting discrete DFE in ayes-ayes (blue), as compared to the DFE in humans (gray) inferred by Johri et al. (2023). Right panel: Comparison of empirical and simulated divergence values for the best-fitting DFE. Green lines represent the mean value, whilst boxes represent the 25th and 75th percentiles.

3.2 | Utilizing Patterns of Exonic Divergence to Infer the DFE

Divergence is an informative summary statistic when inferring patterns of long-term selection, as the general features of the DFE are likely to remain relatively stable over deep evolutionary time. As such, we ran forward-in-time simulations in SLiM (Haller and Messer 2023) to fit observed empirical exonic divergence with a DFE shape consisting of neutral, weakly/moderately deleterious, and strongly deleterious mutational classes. In brief, we simulated a 54.9 million year divergence time of the aye-aye branch (Horvath et al. 2008), assuming a generation time of 5 years (Ross 2003; Louis et al. 2020)—both of which have additionally been recently supported by whole-genome neutral divergence patterns (Soni, Versoza, et al. 2024)—and utilized the estimated demographic model of Terbot et al. (2025) to characterize the recent history of the species. Using our multiple-species alignment to compute the number of divergent sites along the aye-aye branch, we were able to directly compare this empirical observation with the number of fixations accrued in our simulated population during the divergence phase.

As depicted in Figure 3, observed divergence was fit well by a DFE of new mutations characterized by a majority of nearly neutral variants, and a remaining even mix of weakly/moderately and strongly deleterious variants. For comparison, a recent estimate of the DFE from human populations (Johri et al. 2023) has also been included. As shown, humans were characterized by a higher density of neutral variants and a lower density of more strongly deleterious variants relative to aye-ayes, likely consistent with the smaller long-term effective population size of the former. Specifically, while the lower effective population size of humans would be expected to correspond to a reduced efficacy of purifying selection, the strength of selection that any individual mutation experiences is a product of the effective population size and the selection coefficient (i.e., $N_e s$). As such, even if the distribution of selection coefficients (s) were to be identical between species, values of $|N_e s|$ would be expected to be larger in aye-ayes, consistent with the distribution here inferred.

Notably however, this inference in aye-ayes assumes a mutation rate of 0.4e-8/bp/generation, as was directly inferred from pedigree data (Versoza et al. 2025). This pedigree inference was for the youngest parents in the study (9–11 years of age), and a strong parental age effect was observed. Namely, the oldest parents in the study (24–26 years of age) were characterized by a rate of 2.0e-8/bp/generation, with an average rate across the pedigree of 1.1e-8/bp/generation. Given that aye-ayes reach sexual maturity by ~2.5–3 years of age (Winn 1994; Ross 2003), reproduction in the wild likely occurs amongst individuals even younger than the youngest in the pedigree, and given support for the 0.4e-8/bp/generation rate from recent indirect divergence-based inference (Soni, Versoza, et al. 2024), we believe this to be a reasonable estimate for our conversion here. However, if the true rate were to be even lower owing to parents being generally younger throughout the evolutionary history of the species, the inferred DFE would resultingly become more skewed towards nearly neutral variants and thus potentially more similar to the human estimate. It is also noteworthy that long-term mutation rates on the aye-aye branch higher than 0.4e-8/bp/generation become very difficult to reconcile with the fossil-record (see Tavaré et al. 2002; Soni, Versoza, et al. 2024), consistent with the suggestion of generally lower rates in prosimians relative to other primates (see the reviews of Tran and Pfeifer 2018; Chintalapati and Moorjani 2020).

4 | Concluding Thoughts

In this study we have characterized functional divergence in aye-ayes, finding, as expected, that exonic divergence is generally much reduced relative to neutral divergence. As such, no gene or gene set inherently required a positive selection-based explanation, as none were observed to be evolving faster than

the fastest neutral rate. Yet, amongst exons, we also found that the most rapid rates of divergence were restricted to particular functional categories, namely, in genes related to immune and sensory-related functions. This observation is in agreement with previous work across vertebrates for the former, and in primates more specifically for the latter. Employing forward simulations to fit a DFE to observed exonic divergence, we additionally found evidence of an increased proportion of newly arising deleterious variants in aye-ayes relative to humans, likely related to their larger estimated long-term effective population size. These findings also generally support a relatively low mutation rate in aye-ayes compared to other primates, as has been proposed both from indirect neutral divergence as well as from direct pedigree-based inference.

Author Contributions

Vivak Soni: formal analysis (lead), investigation (equal), methodology (equal), software (equal), validation (equal), visualization (equal), writing – original draft (equal). **Cyril J. Versoza:** data curation (lead), investigation (equal), methodology (equal), resources (equal), software (equal), validation (equal). **Susanne P. Pfeifer:** conceptualization (equal), data curation (lead), formal analysis (equal), funding acquisition (lead), investigation (equal), methodology (lead), project administration (lead), resources (equal), supervision (lead), validation (equal), visualization (equal), writing – original draft (equal). **Jeffrey D. Jensen:** conceptualization (lead), formal analysis (equal), funding acquisition (lead), investigation (equal), project administration (lead), supervision (lead), writing – original draft (equal).

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Data Availability Statement

All scripts to generate and analyze simulated data, as well as results from selection scans, are available at the GitHub repository: https://github.com/vivaksoni/aye_aye_mutational_fitness_effects.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.