Genomic erosion across avian lineages in the context of their evolutionary history

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Abstract

Loss of genetic diversity threatens species survival, yet its dynamics and impacts can vary widely across species depending on their evolutionary histories, life-history traits, and demographic trajectories. To investigate these differences, we analysed the genomes of three species that experienced extreme and well-documented population bottlenecks, the Mauritius parakeet, the Mauritius kestrel, and the pink pigeon, and compared them to 36 species spanning the avian phylogeny with varied IUCN Red List statuses. For each species, we assessed nucleotide diversity, genetic load, and inbreeding coefficients based on runs of homozygosity (F_{ROH}). We found a negative correlation between nucleotide diversity and F_{ROH}, but neither metric was a good predictor of the species' Red List status. Rather, the effective population size to census size ratio (Ne/Nc) showed a strong correlation to Red List status. Species with larger historical effective population sizes showed greater heterozygosity but carried a higher heterozygous load, highlighting the importance of historical demography for contextualising species' vulnerability to genomic erosion. We also found significant differences in genetic load between taxonomic groups (parrots, pigeons and falcons), possibly due to differences in life-history traits and demographic histories, underscoring the importance of interpreting genomic erosion dynamics in an evolutionary context. By anchoring our study on three evolutionarily divergent endangered species from Mauritius, we show how multispecies comparisons can contextualize extreme bottlenecks within a broader evolutionary framework, thereby identifying both general patterns of genomic erosion and species-specific vulnerabilities.

Introduction

Genetic diversity is an essential component of a species' ability to adapt and persist under changing environmental conditions (Spielman et al. 2004; Allendorf et al. 2013; Kardos et al. 2021). For small or isolated populations, maintaining genetic diversity is particularly challenging, as reduced effective population size (Ne) diminishes the efficacy of natural selection, intensifies genetic drift, and leads to increased inbreeding. These processes ultimately lead to genomic erosion, characterised by reduced genetic diversity and the accumulation or fixation of deleterious mutations (van Oosterhout et al. 2022). As inbreeding becomes more frequent, recessive deleterious mutations are increasingly exposed, intensifying negative effects and leading to inbreeding depression (Charlesworth and Willis 2009; Blomqvist et al. 2010; Hasselgren et al. 2021). These processes collectively reduce fitness and adaptive potential, heightening vulnerability to environmental changes and threatening the long-term persistence of the population (Blomqvist et al. 2010; Hasselgren et al. 2021; Jackson et al. 2022; van Oosterhout et al. 2022; Jeon et al. 2024). Additionally, even populations that have partially recovered demographically retain the genetic legacy of past bottlenecks, known as "drift debt", which manifests as a time lag between population decline and loss of genome-wide variation (Dussex et al. 2023; Gilroy et al. 2017; Pinto et al. 2024). Recent analyses have shown that species are losing genetic diversity worldwide (Exposito-Alonso et al. 2022; Shaw et al. 2025), highlighting the need for cross-species comparisons to improve our understanding of genomic erosion in biodiversity conservation.

With the continuous and rapid production of genomic data for wild species worldwide, conservation genomics can now take advantage of high-resolution tools to assess genetic diversity and genetic load (Lewin et al. 2018; Wright et al. 2020; van Oosterhout et al. 2022; Theissinger et al. 2023). For species at risk of extinction, such insights are critical for guiding conservation interventions aimed at reducing genetic load and enhancing population viability (e.g., vaquita (Morin et al. 2021), kākāpō (Dussex et al. 2021), pink pigeon (Speak et al. 2024)). Genome-wide comparisons can also provide insights into an endangered species'

susceptibility to introgression from closely related species (Rieseberg 2001; Serrato-Capuchina and Matute 2018; Fontdevila 2019), and opportunities for genetic rescue (Whiteley et al. 2015; Bell et al. 2019). Additionally, using genomic resources across multiple species within a comparative framework may provide valuable insight into how evolutionary and demographic histories shape genomic patterns over both short and long timescales (Grueber 2015). This approach can elucidate how evolutionary history interacts with genomic traits, such as genetic diversity, genetic load, or structural variations, to influence species' long-term viability and extinction risk.

The Mauritius parakeet (*Alexandrinus [Psittacula] eques*), Mauritius kestrel (*Falco punctatus*), and pink pigeon (*Nesoenas mayeri*) exemplify how species can recover demographically from near extinction but remain genetically imperilled. These birds, endemic to Mauritius—a species-rich archipelago in the Indian Ocean that has witnessed over 100 species extinctions in recent centuries (Florens 2013)—experienced some of the most extreme population bottlenecks ever recorded in wild populations. Only four Mauritius kestrels remained by 1974, the pink pigeon declined to ~10 individuals by 1990, and the Mauritius parakeet to just ~20 individuals by 1986 (Jones and Swinnerton 1997; Jones 2013; Jones et al. 2013). Intensive conservation management facilitated their demographic recovery to current free-living adult population sizes of approximately 250 Mauritius kestrels, 500 pink pigeons, and 650 Mauritius parakeets (Jones and Swinnerton 1997; Jones 2010; Nicoll et al. 2021) (Figure 1D). Although these species belong to evolutionarily distant lineages, they underwent similar demographic trajectories of collapse and recovery, providing independent yet comparable case studies to examine the genomic consequences of population decline and recovery across birds.

Despite these recoveries, the legacy of the extreme historical population collapses can jeopardise their long-term viability, as genetic diversity in these species continues to decline due to the accrued drift debt (Tollington et al. 2013; Jackson et al. 2022). These species are also at risk of accumulating an increased genetic load of deleterious mutations, as has been shown in the pink pigeon (Jackson et al. 2022). Beyond genetic challenges, ecological pressures such as habitat loss and degradation persist, compounded by threats like

emerging infectious diseases, which can affect individual fitness and population viability (Tollington et al. 2015).

This study investigates the interplay between genome-wide diversity, genetic load, demographic history, and conservation status across a diverse set of avian species. Using recently generated, high-quality chromosome-level reference genomes for three focal Mauritian species – the Mauritius parakeet, pink pigeon and Mauritius kestrel – we compared them to 36 species spanning the avian phylogeny. Placing these species in a broader phylogenetic context allowed us to distinguish general temporal trends from lineage-specific outcomes and to evaluate how such insights can inform conservation assessments and prioritisation. By further focusing on species within the same orders as the Mauritian taxa, we explored potential lineage-specific differences in genomic metrics to contextualise the genetic risk of the Mauritius species against the backdrop of their phylogenetic background and to inform conservation priorities for other vulnerable birds.

Materials and Methods

Dataset

The reference genomes from three bird species endemic to Mauritius—the Mauritius parakeet (Morales, Groombridge, et al. 2024), the pink pigeon (Morales, Van Oosterhout, et al. 2024), and the Mauritius kestrel (Morales, Norris, et al. 2024)—were recently sequenced and reported. All three genomes were generated from samples collected post-bottleneck, from 2020 to 2021. To build a comparative dataset, 36 additional bird species were selected based on the availability of relatively high-quality reference genomes, prioritizing assemblies with higher scaffold N50, larger average scaffold size, and lower scaffold count within each taxonomic group, while also ensuring a comprehensive representation across the avian phylogeny (Figure 1B). Although no strict thresholds were applied, the majority of selected genomes had scaffold N50 values >2 Mb and >85% of their genome contained within scaffolds >500 kb; a small number of species with slightly lower metrics were included to fill key phylogenetic gaps. Metadata for all 39 species, including current census population sizes, IUCN conservation statuses, and assembly statistics are compiled in Table S1.

Mapping and variant calling

Raw reads used for assembling the reference genomes were downloaded from NCBI (see Table S1 for Assembly IDs) and aligned to the corresponding genomes. NGS short reads were mapped using BWA (v0.7.17) mem (Li and Durbin 2009) with default parameters. Read duplicates were marked with GATK (4.4.0.0) MarkDuplicates (DePristo et al. 2011). PacBio HiFi reads were mapped and sorted using pbmm2 v1.5.0 (https://github.com/PacificBiosciences/pbmm2) with the parameter "--preset HIFI". GATK HaplotypeCaller was used to call variants for each alignment. Only SNPs were kept for further analyses.

Depth

After mapping the raw reads to each reference genome, we estimated the average genome-wide and per scaffold depth in each genome using MosDepth v0.3.3 (Pedersen and Quinlan 2018).

Sex chromosome removal

Each reference genome was mapped to the chicken genome (assembly GRCg6a) with minimap2 v2.1 (Li 2018). Any scaffold mapped to chicken sex chromosomes for more than 20% was treated as potential regions from sex chromosomes and removed for further analyses. Any additional scaffolds annotated in the reference genomes as sex chromosomes were also removed.

Heterozygosity

We estimated genome-wide heterozygosity using ANGSD (Korneliussen et al. 2014). We first obtained genotype likelihoods on scaffolds larger than 500 kb and only considered sites with a depth of depth between ⅓ (-setMinDepth) and two times (-setMaxDepth) the average depth for each sample. We assumed that the reference and ancestral states were the same. We applied the following parameters: -uniqueOnly 1 -remove_bads 1 -only_proper_pairs 1 -C 50 -baq 0 -minMapQ 30 -minQ 20 -setMinDepth $minDP -setMaxDepth $maxDP -doCounts 1 -GL 2 -doSaf 1. Next, we calculated the folded site frequency spectrum (SFS) with realSFS.

To compare different estimations of heterozygosity, we also estimated genome-wide heterozygosity directly from the VCF files with a custom pipeline. First, we divided scaffolds into sliding windows of size 100 kb (with a slide of 50 kb) using bedtools makewindows v2.30.0 (Quinlan and Hall 2010). Next, we obtained the total number of callable heterozygous and total callable genotypes per window using bcftools v1.20 (Danecek et al. 2021), tabix v1.14 (Li 2011), and vcfhetcount from vcflib (Garrison et al. 2022). Genotypes were considered callable if their read depth was between ⅓ and 2 times the average depth per sample and had a minimum genotype quality (GQ) of 30 or reference genome quality (RGQ) of 10, indels and multiallelic sites were excluded. Only non-missing and quality filtered sites (callable) were considered in each window and used as total callable genotypes

in the denominator. Windows with less than 50% callable sites relative to the window size were removed for analysis.

Runs of Homozygosity (ROH)

As some reference genomes were assembled with only Pacbio long reads, and no short-read data are available for the same individual, commonly used methods like ROHan (Renaud et al. 2019) could not be applied to identify ROH. To address this limitation, we developed a custom method for this analysis. Given the varying fragmentation levels of the assemblies (Table S1), for each species, we retained only scaffolds with a minimum size of 5 Mb. As a result, two species (Red-faced mousebird, *Urocolius indicus* and Wilson's storm petrel, *Oceanites oceanicus*) were excluded. We identified ROH based on per-window heterozygosity estimates (see above). Using the R package bedtoolsr (Patwardhan et al. 2019), we concatenated windows with a heterozygosity lower than $5e^{-4}$ bp^{-1} (Figure S1), except for two genomes with exceptionally low average heterozygosity (the flightless cormorant, *Nannopterum harrisi*, and Mauritius kestrel), for which a lower threshold of $1e^{-4}$ bp^{-1} was used. Adjacent homozygous regions were merged if separated by a gap shorter than 100 kb, and only ROHs with a minimum size of 500 kb were retained. The inbreeding coefficient (F_{ROH}) was calculated as the ratio of the total length of ROH segments to the total length of the analyzed genome (scaffolds > 5 Mb). We also estimated heterozygosity for the analyzed scaffolds per species, both including and excluding ROHs. To obtain the heterozygosity outside ROHs, we subtracted the windows that fall within a ROH for the estimation.

To validate our custom method, we compared our ROH estimates with those from ROHan for five species for which short-read data were available. ROHan was employed using a window size of 100Kb and a rohmu of $5e^{-4}$ to mimic the parameters used in the custom method.

Genetic load

To compare genetic load across species, we used Combined Annotation-Dependent Depletion (CADD) score annotations (Kircher et al. 2014). CADD score integrates multiple

genome annotations to rank the deleteriousness of any possible single-nucleotide variant, including conservation metrics (e.g., GERP, Cooper et al. 2005), regulatory and transcript information, and protein-based scores (e.g., SIFT, Ng and Henikoff 2003). While CADD annotation of deleteriousness of mutations is generally consistent with regular functional annotation based on protein-coding information (Fontsere et al. 2025) such as SnpEff (Cingolani et al. 2012), CADD can also rank mutations in the introns and intergenic regions. We used the CADD score calculated for chicken genome (Groß et al. 2020) and transferred them to evolutionary conserved regions of the genome (ultraconserved elements, UCEs) in our target species. UCEs are highly conserved in vertebrates over a long evolutionary period, especially in birds (Cummins et al. 2024). Though mainly in non-protein coding regions (introns or lncRNA genes), mutations in UCEs, especially with a high CADD score, are expected to have a strong deleterious effect (Speak et al. 2024). We extracted UCE regions and corresponding flanking regions from each reference genome and from the chicken genome (GRCg6a), and performed a multi-species alignment for each UCE, using the recommended pipeline of Phyluce v1.7.3 (Faircloth 2016)see Figure S2 for detailed pipeline). Each genome was converted from fasta to 2bit format using UCSC FaToTwoBit (Casper et al. 2018). The UCE probe file was downloaded from https://github.com/faircloth-lab/uce-probe-sets/tree/master/uce-5k-probe-set, and used to extract and validate UCE regions per species. The CADD score file of the chicken genome was downloaded from https://osf.io/c97ez. For each species, CADD scores were lifted from the chicken genome for homozygous (relative to chicken) and heterozygous sites across UCE regions with customised scripts partially adapted from LoadLift (Speak et al. 2024). Heterozygous sites were extracted from VCF files and filtered using bcftools with the same parameters used for heterozygosity estimations (see above). Only heterozygous sites with one allele equal to the corresponding site in the chicken genome were kept for further analyses.

To quantify genetic load in each genome, we counted the number of heterozygous sites and homozygous substitutions with CADD scores $\geqslant$ 20 in UCE regions, representing the top 1% most deleterious sites in the chicken genome. To control for the evolutionary distance to the chicken among species, we rescaled the counts of sites with CADD scores $\geqslant$ 20 by dividing

them with the counts of substitutions with CADD scores < 3, the latter representing nearly-neutral sites. The rescaled ratios of heterozygous sites and homozygous substitutions were used as inference of heterozygous and homozygous load. We distinguished these two classes because, according to theory (Bertorelle et al. 2022), deleterious homozygous positions are expressed, approximating the realised load, whereas (recessive) heterozygous positions are not expressed, approximating the masked load. However, in the absence of precise estimates of dominance coefficients, these are only approximations, and we therefore prefer to refer to them as the homozygous and heterozygous load.

To account for potential lineage-specific adaptive substitutions that have occurred since the divergence with chicken, homozygous substitutions that were shared by 20 or more species were excluded, as these are more likely to reflect long-term adaptive changes rather than harmful mutations. To further confirm that we were correctly retaining sites that have not changed since the divergence with chicken, we compared three species (saker falcon, *Falco cherrug*; budgerigar, *Melopsittacus undulatus*; and rock pigeon, *Columba livia*) included in the phylogenetic tree by (Feng et al. 2020), to their closest reconstructed ancestral nodes in the tree, which correspond to genus or family level. The command “hal2fasta” from HAL tools v2.3 (Hickey et al. 2013) was used to extract the sequences of the ancestral nodes from the genome-level alignment from this phylogenetic tree of 363 bird species. We extracted the reconstructed full sequences from the closest ancestral nodes and cut them into 200-bp short sequences with 20-bp step sliding windows. The reads were mapped to the chicken genome with BWA. For each species, we confirmed that homozygous substitutions had at least one ancestral read mapped to the chicken genome, and the ancestral state matched the chicken sequence. Homozygous substitutions with CADD scores above 20 are concentrated at the terminal branches of the phylogenetic tree of birds (Figure S3), indicating that they are more likely to be deleterious substitutions than lineage-specific adaptations. Although we cannot rule out lineage-specific adaptive substitutions when measuring homozygous load, focusing on sites with CADD > 20 within the most conserved regions provides a higher likelihood of targeting truly deleterious variation (Rentzsch et al. 2019; Speak et al. 2024; Fontsere et al. 2025) (Figure S3). As above, the counts of

substitutions with CADD scores < 3, considered as nearly-neutral sites, were used to rescale the counts of sites with CADD scores ⩾ 20.

Demographic history

We inferred historical fluctuations of effective population size (Ne) for each species using PSMC (Li and Durbin 2011) with the parameters "-N30 -t5 -r5 -p 1+1+1+1+30*2+4+6+10" (Hilgers et al. 2025) and estimated the harmonic Ne mean from 10 kya to 100 kya for further analyses. The generation times and mutation rates used for PSMC can be found in Table S1. Generation times were retrieved from IUCN Red List (IUCN 2024), and the source of mutation rates were listed in Table S1.

Statistical and phylogenetic comparative analyses

To explore the relationships among genome-wide heterozygosity, F_{ROH}, Ne, and genetic load across species while accounting for phylogenetic signals, we reconstructed the phylogenetic relationships among the studied species and incorporated this inference into two statistical frameworks. The phylogeny was reconstructed from a subset of the UCE dataset described above (see *Genetic Load* section). Using the software AMAS (Borowiec 2016), we selected and concatenated 1,526 UCE sequences, retaining only loci with ≤2% missing data and >30% parsimony-informative sites. As previously published phylogenies did not include the target Mauritius species, we used IQ-TREE (Minh et al. 2020) to infer a maximum-likelihood tree using the edge-linked partition model (Chernomor et al. 2016) constraining deeper relationships based on the topology from Stiller et al. (2024) This approach allowed us to integrate our focal species into a well-supported, coalescent-informed avian phylogeny. Using this inferred phylogenetic tree, we assessed different univariate models with a phylogenetic generalised least squares (PGLS) approach (Freckleton et al. 2002). These analyses were conducted using the R packages *ape* (Paradis et al. 2004; Paradis and Schliep 2019), *caper* (Orme et al. 2023) and *nlme* (Pinheiro et al. 2014). When PGLS indicated a non-zero lambda (λ) value, suggesting a significant phylogenetic signal, we further examined these effects using a Bayesian phylogenetic

generalised linear mixed model (pGLMM) (Hadfield and Nakagawa 2010). In this framework, the phylogenetic relationship among species was modelled as a random effect. pGLMM analyses were performed using the R packages *ape* and *MCMCglmm* (Hadfield 2010).

Genomic synteny

We inferred multi-genome synteny for chromosome-level reference genomes for pigeons (three species in Columbidae), parrots (five species in Psittaciformes), and falcons (six species in *Falco*) separately using ntSynt v1.0.2 (Coombe et al. 2024) with the divergence range (-d) set to 10. Synteny results were visualised using scripts from ntSynt based on the R package *gggnomes* (Hackl et al. 2024).

Identification of Transposable Elements

To annotate repetitive elements (RE) in the genomes of the pink pigeon, Mauritius kestrel, and Mauritius parakeet, we produced *de novo* libraries of RE for each species using RepeatModeler2 (Flynn et al. 2020). We combined the *de novo* libraries with previously published manually curated libraries of RE from the Collared flycatcher (*Ficedula albicollis*) and Blue-capped cordon-bleu (*Uraeginthus cyanocephalus*) from Storer et al. (2021), and from the Emu (*Dromaius novaehollandiae*), Anna's hummingbird (*Calypte anna*), and Kākāpō (*Strigops habroptilus*) from Peona et al. (2021). Using the resulting custom libraries, we annotated the RE from the genomes using RepeatMasker version 4.0.8 (Smit et al. 2015). We repeated this process for three, five and four additional species of Columbiformes, Falconiformes and Psittaciformes, respectively, to enable comparisons of proportions of RE across species.

Results

Genome-wide diversity and inbreeding

The sequencing depth across the dataset ranged between 15x and 96x (mean = 46, SD = 19). We estimated genome-wide heterozygosity with both genotype likelihoods in ANGSD and by SNP-calling, resulting in very similar estimates (adjusted R^2 = 0.78; Table S1, S2, Figure S4). We used heterozygosity estimates from ANGSD for all subsequent analyses, except for the

estimation of ROHs (see Material and Methods). Neither heterozygosity or F_{ROH} estimates showed significant correlation with the quality of the genomes (e.g., N50) or depth (Figure S5). Our in-house method produced consistent ROH results to those from ROHan (Figure S6), validating our approach.

Consistent with recent demographic change, genome-wide heterozygosity showed a strong negative correlation with inbreeding coefficient F_{ROH} (Figure 1C; PGLS: $\lambda = 0$, $R^2 = 0.46$, $F_{1\text{-}28} = 26.56$, $p < 0.001$). Samples with domestic or pet origins (Figure 1C, Table S1) are outliers with higher F_{ROH} compared to wild samples with similar levels of heterozygosity (Figure 1C), and higher heterozygosity outside of ROH regions compared to genome-wide heterozygosity (Figure S7); we therefore excluded them from further analyses. IUCN Red List status did not correlate with genome-wide heterozygosity, F_{ROH} (Figure S8) or genetic load (Figure S9).

Comparing historical Ne (between 10 kya and 100 kya estimated from PSMC) to current census size (Nc) reveals the magnitude of recent demographic change, with elevated values indicating more abrupt declines. The Ne/Nc ratio (Log(Ne)/Log(Nc)) was significantly lower (Wilcoxon two-sample test $p < 0.001$) in non-threatened species (Least Concern, mean = 0.91, SD = 0.23) compared to threatened species (remaining IUCN status categories, mean = 1.49), yet more varied (SD = 0.52). Ne/Nc ratio showed a strong linear correlation with IUCN status (Figure 1D, GLM: $R^2 = 0.43$, $F_{1\text{-}24} = 12.49$, $p < 0.001$), when numerically coded from 0 Critically Endangered to 4 Least Concern. To disentangle the contribution of each component, we tested log-transformed Nc and Ne separately (Figure S8). In these tests, only Nc was significant ($p = 0.0186$, $R^2 = 0.27$), whereas Ne was not. However, the Ne/Nc ratio explained more variance than either component alone ($p < 0.001$, $R^2 = 0.43$), indicating that the ratio captures additional, meaningful variation. Moreover, the Ne/Nc ratio reveals discrepancies that are not evident from Nc alone. For example, the pink pigeon and the whooping crane (*Grus americana*) both exhibit unusually high Ne/Nc ratios (2.19 and 2.59, respectively) compared to other species within the same IUCN categories (Vulnerable and Endangered, respectively). These elevated ratios point to a history of intense demographic decline despite currently recovered census sizes, underscoring that the severity of underlying genomic erosion is not apparent with Nc or Ne alone.

Historical demographic trends (Ne) have a significant positive correlation with genome-wide heterozygosity (Figure 2A; PGLS: $\lambda = 0$, $R^2 = 0.47$, $F_{1\text{-}22} = 19.65$, $p = 2.1e^{-4}$). In contrast, F_{ROH} showed no significant correlation with historical Ne (Figure 2D; PGLS: $\lambda = 0$, $R^2 = 0.11$, $F_{1\text{-}22} = 2.82$, $p = 0.11$). This lack of correlation is not surprising as the inbreeding coefficient is expected to have a nonlinear relationship with Ne (Reed et al. 2003), because inbreeding increases rapidly in small populations but approaches equilibrium asymptotically as Ne grows. Furthermore, our analysis focused only on long ROHs (> 500 Kb), which reflect population history within tens to hundreds of generations ago.

Species with higher genetic diversity or a lower inbreeding coefficient tended to carry a higher heterozygous load, calculated as the corrected ratio of heterozygous sites with a CADD score above 20 (Figure 2B; PGLS: $\lambda = 0$, $R^2 = 0.61$, $F_{1\text{-}30} = 45.99$, $p = 1.6e^{-7}$, and Figure 2E; PGLS: $\lambda = 0$, $R^2 = 0.19$, $F_{1\text{-}28} = 6.74$, $p = 0.015$). In contrast, estimated homozygous load, calculated as the corrected ratio of homozygous substitutions with a CADD score above 20, showed a statistically significant but weak association with heterozygosity (Figure 2C; PGLS: $\lambda = 0.81$, $R^2 = 0.16$, $F_{1\text{-}30} = 5.61$, $p = 0.0245$), and no association with F_{ROH} (Figure 2F; PGLS: $\lambda = 0.86$, $R^2 = 0.02$, $F_{1\text{-}28} = 0.61$, $p = 0.4424$). Because $\lambda \approx 0.8$ indicated phylogenetic signal in the residuals, we refitted these models using a phylogenetic GLMM (pGLMM) with a random effect for shared ancestry. This model recovered the same fixed-effect pattern, as heterozygosity remained significant and F_{ROH} non-significant, but revealed that phylogenetic variance was negligible compared to residual variance ($\sigma^2\_phylo \approx 4.1\times10^{-14}$; $\sigma^2\_species \approx 1.0\times10^{-14}$; Table S3). Thus, although residuals appeared phylogenetically structured in the PGLS, shared ancestry explained little additional variation once predictors were included, indicating that phylogenetic relatedness is not a major determinant of homozygous load variation. Although statistically significant, the association between homozygous load and heterozygosity remained weak, and the absence of a positive correlation between homozygous load and F_{ROH} was unexpected (Kyriazis et al. 2025). This pattern may reflect the influence of deep-time factors such as lineage-specific mutation rates, adaptive

substitutions, or life-history differences. Moreover, because only one individual per species was analyzed, it remains difficult to distinguish fixed substitutions accumulated through deep phylogenetic time from genuinely deleterious homozygous alleles (see Discussion).

Genomic features within and across taxonomic groups

Despite similar distribution ranges and histories of population decline in the past decades (Figure 1A), the three Mauritius species showed contrasting levels of genetic heterozygosity, inbreeding coefficients (Figure 1C, 3AB), and homozygous load (Figure 3C). The Mauritius kestrel had the lowest heterozygosity of all samples (8.33×10^{-5} het x bp^{-1}) and the second highest F_{ROH} (0.71) among the wild species included in this study, with 50% of its genome in very long ROHs (>10 Mb), as evidence of sustained recent inbreeding after recovering demographically from a bottleneck of only four individuals. The Mauritius parakeet had the second lowest heterozygosity (8.07×10^{-4} het x bp^{-1}) among parrots, closely following another extremely bottlenecked species, the critically endangered Kākāpō (*Strigops habroptilus*). However, the Mauritius parakeet's F_{ROH}, while high (0.40), is lower than that of the Kākāpō (0.69), with 24.7% of the Mauritius parakeet's genome in ROHs longer than 10 Mb, evidence of their extreme bottleneck of ~12 individuals. The pink pigeon exhibits a heterozygosity of 2.38×10^{-3} het x bp^{-1}, the lowest among the analysed pigeons, but higher than more than half of the species included in the study and nearly 30 times greater than the Mauritius kestrel. Additionally, the pink pigeon showed an F_{ROH} of 0.26, with 12.3% of its genome in ROHs of a length longer than 10 Mb (Figure S7).

Reflecting a deeper evolutionary process, the differences in genetic diversity of Mauritius species were associated with the differences between their taxonomic groups (Figure 3). Falcons exhibit lower heterozygosity (one-side Wilcoxon test p=0.07) than the other two taxonomic groups. Likewise, falcons carry 23.7% less homozygous load than the parrots (p=0.001) and 29.7% less than the pigeons (p=0.005). No significant difference of F_{ROH} found between taxonomic groups. Within their respective taxonomic groups, the three Mauritius species showed the lowest genome-wide heterozygosity (Figure 3A) and the highest F_{ROH} (Figure 3B). Genetic diversity estimates carry the signal of ancestral population size, as the

three Mauritius species had relatively low population sizes within their respective taxonomic groups (Figure 3D). However, the pink pigeon had a larger historical population than most studied species, including falcons and parrots, which is reflected in its higher heterozygosity compared to the average levels in the other taxonomic groups (Figure 3A). This reveals the importance of considering genetic diversity within the context of a species' long-term evolutionary history and taxonomic group. Note that all species that we have included from Falconiformes are from the same genus, *Falco*, with a divergence time of 12 million years (Kumar et al. 2022), which could explain the small deviations for heterozygosity and homozygous load, whereas the divergence time of the study Columbiformes and Psittaciformes species was roughly 16 and 55.6 million years, respectively (Figure S10).

We examined synteny and repetitive element content in the three Mauritius species to ensure that their differences did not confound interspecific comparisons. All exhibited conventional avian genome structures, with conserved synteny to their closest relatives (Figure S10) and typical repetitive element proportions (15–20%; Figure S11; Hughes & Piontkivska 2005), consistent with previous reports of lower repeat content in birds relative to mammals.

Discussion

By analysing 39 avian genomes spanning diverse taxonomic groups, we show that the dynamics of genetic diversity, genetic load, and inbreeding are shaped by both recent demographic changes and deep evolutionary history. While the genomic effects of recent population decline have been well documented (van der Valk, Diez-del-Molino, et al. 2019; Hasselgren et al. 2021; Khan et al. 2021; Cavill et al. 2024), we show how deep demographic history also exerts a long-lasting influence on patterns of genomic erosion. As a result, species from different taxonomic groups exhibit distinct levels of heterozygosity and genetic load, underscoring the need to account for both demographic history and phylogenetic context when comparing genomic metrics. Using three Mauritian species as focal case studies, we illustrate how comparative analyses can uncover conservation-relevant patterns that would be overlooked in single-species studies. Despite challenges in standardizing

genomic metrics across diverse taxa, our findings demonstrate how evolutionary history constrains the interpretation of genomic metrics widely used in conservation, highlighting the value of comparative frameworks for advancing biodiversity conservation.

Conservation status is shaped by recent and long-term history

Genetic diversity has been considered a classical indicator for population resilience and risk of extinction (Breed et al. 2019; DeWoody et al. 2021; Teixeira and Huber 2021; Jeon et al. 2024). Consistent with this expectation, we observed a negative correlation between genome-wide heterozygosity (a proxy of genetic diversity), and F_{ROH} (a proxy of inbreeding) (Figure 1C), reflecting intensified inbreeding and genetic drift in recently small populations (Brüniche-Olsen et al. 2018; Grossen et al. 2020). Reference genomes sequenced from domestic or pet samples had elevated F_{ROH}, despite relatively high heterozygosity outside ROHs, highlighting how recent inbreeding can bias genome-wide heterozygosity estimates and the importance of checking sample origin when using public genomic data (Figure S7).

Over longer evolutionary timescales (10,000 to 100,000 years ago), genetic diversity was strongly correlated with historical population size (Figure 2A), indicating that present-day genomic variation continues to reflect ancient demographic history. This suggests that species' genetic susceptibility to future habitat and environmental change is associated with its ancient demographic history, even prior to the accelerated recent environmental changes induced by human activity (Tan et al. 2023). F_{ROH} on the other hand, did not show a strong correlation with the historical population size (Figure 2D), as it should instead reflect the effect of recent demographic change (Ceballos et al. 2018). Consequently, joint monitoring of demographic, environmental, and genetic change – using complementary genomic metrics – is essential for evaluating short- and long-term risks. This is particularly important for species with historically low genetic diversity, as they may remain more vulnerable to future environmental shifts even if current population sizes appear stable (Ellstrand and Elam 1993; van der Valk et al. 2019; Brüniche-Olsen et al. 2021; Liu et al. 2025; Willi et al. 2006).

Data on modern census population sizes (Nc) are undoubtedly crucial in conservation assessments (Shaffer 1981; Lande 1988; Willi et al. 2006; Frankham et al. 2014), with the rate of demographic decline being one of the major factors considered by the IUCN Red List rating (Frankham et al. 2014; McNeely et al. 1990). However, the Ne/Nc ratio, reflecting the balance between long-term genetic diversity and current population size, can also have a prominent role in conservation assessments (Frankham 1995; Kalinowski and Waples 2002). Elevated Ne/Nc ratios based on current Ne likely reflect genetic erosion that has already occurred, while elevated ratios based on historical Ne reflect recent demographic declines that have not yet resulted in a proportional loss of genome-wide diversity. The latter ratio reflects the time-lag between population decline and genetic diversity loss (Gargiulo et al. 2024), resulting from the drift debt (Gilroy et al. 2017; Dussex et al. 2023; Pinto et al. 2024; Liu et al. 2025), and serves as an early warning sign of an imminent population collapse (Amos and Balmford 2001; Wilder et al. 2023). In this study, we use historical Ne estimated via PSMC, enabling a comparison across many species without the need for population-level sampling. We find that elevated Ne/Nc ratios are associated with higher IUCN risk categories (Figure 1D). Although the correlation between IUCN status and Ne/Nc ratio is mainly driven by Nc (Figure S8), as IUCN rating is partly based on current population sizes, the Ne/Nc ratio has a stronger correlation to IUCN status than Nc alone (Figure S8), suggesting that it captures additional demographic-genetic dimensions. Notably, species such as the pink pigeon and whooping crane exhibit unusually high Ne/Nc ratios relative to their IUCN category, revealing discrepancies that may signal severe ongoing genomic erosion, as a result of a sharp historical demographic decline despite demographic recovery. This highlights the value of integrating genomic data into conservation assessments to better capture long-term genetic threats, as previously discussed for these species (Jackson et al. 2022; Fontsere et al. 2025). One potential caveat is the uncertain sampling time of the biological materials used to develop the reference genomes, which could bias our analyses of IUCN status, since nine out of 39 species had status changes within the past 20 years. Our findings indicate that the Ne/Nc ratio, even when based on historical Ne, is not only a meaningful indicator of conservation status (Figure 1D), but also a potential flag for

discrepancies between genomic erosion and current IUCN status. This underscores the importance of understanding and incorporating long-term demographic history into conservation assessments to better capture latent genetic risks.

Interpreting genetic load patterns across species

Our results highlight that the relationship between diversity and load must be interpreted in light of evolutionary timescale and demographic context. We found a strong positive correlation between the relative number of heterozygous deleterious sites – a proxy for masked load – and genome-wide heterozygosity (Figure 2B). Therefore, similarly to genetic diversity, masked load is also shaped by demographic history. Population decline often leads to the expression of masked genetic load, driven by genetic drift and reduced purging (van der Valk, Diez-del-Molino, et al. 2019; Dussex et al. 2023). With habitat loss predicted to intensify in the near future, resulting in accelerated population declines and loss of genetic diversity (Exposito-Alonso et al. 2022), species with currently higher diversity may face rapid exposure of these deleterious mutations before effective purging can occur, increasing the risk of fitness reductions and jeopardising population viability (van Oosterhout et al. 2022). Therefore, estimating heterozygous load remains highly informative, as it integrates demographic and selective history and may ultimately help predict population fitness once empirical links are established.

Our results reveal that species with lower genetic diversity exhibit reduced homozygous load (Figure 2C). This contrasts population-genetics expectations that small low-diversity populations accumulate more deleterious mutations through genetic drift (Kimura et al. 1963; Bertorelle et al. 2022; Robinson et al. 2023), and with empirical studies (Khan et al. 2021; Wang et al. 2023), based on standing variation under constant selection coefficients. Several factors may explain this apparent discrepancy.

First, our estimates rely on single individuals per species, which limits the resolution of polymorphic deleterious alleles and sensitivity to recent demographic effects. As we are comparing single genomes per species spanning >60 million years of avian evolution, our estimates of homozygous load primarily reflect long-term evolutionary accumulation of

substitutions in conserved regions, a mixture of mildly deleterious and lineage-specific adaptive changes (Grossen et al. 2020; Dussex et al. 2023; Kardos et al. 2023; Wang et al. 2023). The difficulty of distinguishing between these categories likely weakens cross-species correlations with diversity and inbreeding. Improved ancestral state inference and population-level sampling will be required to disentangle fixed deleterious substitutions from adaptive changes and to accurately quantify realized load within species.

Moreover, differences in life-history traits may modulate the rate of deleterious allele accumulation. Species with long generation times experience fewer generations over comparable evolutionary periods, potentially slowing the accumulation of weakly deleterious alleles, whereas species with short generation times but large population sizes may maintain more efficient purifying selection. However, these relationships are complex, as generation time often covaries with population size and other ecological factors (Chao and Carr 1993; Sæther et al. 2005; Plough 2016).

Finally, because our lifted-over CADD annotations are based on noncoding regions ultraconserved across birds, they provide reliable indicators of evolutionary constraint, but the direct functional consequences of mutations in these regions remain incompletely understood. Therefore, our estimates should be interpreted as comparative indicators of evolutionary constraint rather than as absolute measures of fitness impact.

Lineage-specific patterns across taxonomic groups

The three Mauritian species exhibit low genetic diversity and high inbreeding (F_{ROH}) within their respective taxonomic groups, consistent with their severe population bottlenecks. These metrics highlight an ongoing risk of inbreeding depression despite their successful conservation management (Jones et al. 1995; Jones and Swinnerton 1997; Tollington et al. 2013; Nicoll et al. 2021). Although absolute levels of heterozygosity and homozygous load differ among the three species, the similarly high F_{ROH} values indicate comparable levels of recent inbreeding across the Mauritian taxa (Figure 3B), suggesting the need for future population monitoring and management focused on reducing the risk of inbreeding.

Placing these patterns in a broader evolutionary context, the contrasts among taxonomic groups reveal the interplay between long-term demographic history and deep-time evolutionary processes. Falcons as a group show consistently low heterozygosity and homozygous load, whereas parrots and pigeons display higher overall genomic diversity. This pattern mirrors variation in ancestral effective population size inferred from PSMC trajectories (Figure 3D), indicating that ancient demography has left a strong imprint on present-day diversity. By contrast, F_{ROH} estimates based on long ROHs show similar values across taxonomic groups (Figure 3B, pairwise Wilcoxon tests were not significant), as these capture recent inbreeding rather than long-term demographic history.

Life-history traits likely mediate these taxonomic differences. Parrots have long generation times, low reproductive rates, and high parental investment (Jones and Swinnerton 1997; Jones 2010; Jones et al. 2013), making them particularly vulnerable to genomic erosion. These traits slow the recovery of genetic diversity after bottlenecks and exacerbate the accumulation of homozygous load. In contrast, pigeons have shorter generation times and higher reproductive rates (Jones 2013), facilitating faster recovery and preserving higher genetic diversity despite similar population collapses. Falcons exhibit intermediate traits, with low reproductive rates but shorter generation times and the ability to disperse to new environments (Jones et al. 1995; Cartwright et al. 2014; Nicoll et al. 2021), which can limit genetic drift and inbreeding but may not fully mitigate the effects of historically small population sizes.

Together, these results illustrate that genomic erosion cannot be interpreted outside its phylogenetic and life-history context, and they highlight the need to deepen our understanding of how life-history traits influence genetic diversity (Germain et al. 2023) and load.

Future directions to benefit conservation genomics

As whole-genome data become more accessible (Feng et al. 2020; Stiller et al. 2024), the integration of genomic-derived metrics (e.g., demographic reconstructions, heterozygosity, F_{ROH}, Ne/Nc) with demographic, ecological and environmental data will substantially

improve conservation assessments and planning. Expanding the availability and taxonomic breadth of reference genomes will further increase the utility of genomic resources in conservation biology (Grueber 2015; Supple and Shapiro 2018; Mc Cartney et al. 2024). Given the substantial differences of genome-wide features observed between groups (Figure 3, S10, S11), it is advantageous, even in the absence of a species-specific reference genome, to identify a closely related reference genome to minimize mapping bias and improve inference accuracy (Prasad et al. 2022). However, population-level data remain essential, as a single individual cannot fully represent the genetic diversity of an entire species. Incorporating population-level data also allows for more robust estimates of realised genetic load by leveraging site frequency distributions (Grossen et al. 2020; Bertorelle et al. 2022).

Although the importance of genetic diversity is well established, understanding the fitness effects of deleterious alleles is central to predicting species' adaptive potential and persistence (Kardos et al. 2021). Future progress will require combining genomic data with direct fitness measures, temporal datasets tracking load dynamics, and improved methods to identify deleterious mutations (Bosse et al. 2019; van der Valk, de Manuel, et al. 2019; Bertorelle et al. 2022; Fontsere et al. 2025).

Integrating demographic history changes is also critical for interpreting genetic diversity trends. While genomic inference with modern samples reveals population history in both the long term (Ne) and short term (recent inbreeding with F_{ROH}), historical genetic data provides critical pre-decline information in accurately assessing trends in population size and genetic load in a more recent, crucial time scale (van der Valk et al. 2019; Femerling et al. 2023; Cavill et al. 2024; Dehasque et al. 2024; Silver et al. 2024; Fontsere et al. 2024). Such data are invaluable for identifying rapid losses of genetic diversity and increases in load, which may otherwise go undetected until demographic impacts become severe (Díez-del-Molino et al. 2018).

Although the relationships among genetic diversity, demographic history, and deleterious variation are well established in theory, our findings underscore the importance of considering evolutionary history, demographic processes, and life-history traits when applying genetic measures in conservation. Our comparative analyses indicate that low

genetic diversity or high load does not carry equivalent implications across taxa, and that evolutionary context is essential for interpreting genomic risk. Comparative analyses across lineages provide a unique chance to evaluate how recent demographic collapse interacts with deep-time history, helping to refine expectations for genetic recovery and to better contextualize genomic data for guiding future conservation strategies in an era of rapid environmental change.

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Data Availability

The scripts used in this study are available on GitHub: PachecoMC/CompConGen.

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Figure 1. Demographic trajectory of the three Mauritius species and phylogenetic, genetic diversity, and Ne/Nc ratio distribution of the 39 species used in this study. A Demographic trajectory of the wild population derived from field monitoring of adult individuals over time. Note that the three species have been monitored in different ways so presented trends are approximations of their total numbers. **B** Phylogenetic relationship among the avian orders sampled in this study. Tree topology follows Stiller et al. (2024) and branch lengths are not time-calibrated and do not reflect divergence times. The orders of the three Mauritius species are marked in bold. Each circle represents a sampled species within its respective order. The colour and initials indicate the IUCN Red List category of each species. **C** Genome-wide heterozygosity (circles) and runs of homozygosity-based inbreeding coefficient (F_{ROH}; diamonds) for each species. Colour coding corresponds to IUCN Red List categories. Domestic species are denoted with lighter colours and in grey (outer line in the dots and in their name in x-axis). **D** Correlation between the Ne/Nc ratio after log-transform (Log(Ne)/Log(Nc)) and IUCN Red List categories. The effective population size (Ne) was estimated as the harmonic mean of PSMC values from 10 kya to 100 kya, whereas the census population size (Nc) was obtained from IUCN Red List data. Photo credits: Samantha Cartwright for the Mauritius kestrel (*Falco punctatus*), Jacques de Speville for the Mauritius parakeet (*Alexandrinus eques*), and Gregory Guida for the pink pigeon (*Nesoenas mayeri*).

Figure 2. Comparison between genetic diversity metrics, genetic load, and effective population sizes. The dashed line represents the linear correlation when $p<0.05$. **A** Correlation between genome-wide heterozygosity and effective population size (Ne), with Ne estimated as the harmonic mean of PSMC values between 10 kya and 100 kya. **B** Correlation between heterozygous load and genome-wide heterozygosity. Heterozygous load is defined as the ratio of heterozygous substitutions with CADD > 20 to homozygous substitutions with CADD < 3. **C** Correlation between homozygous load and genome-wide heterozygosity. Homozygous load is defined as the ratio of counts of filtered homozygous substitutions with CADD > 20 and the number of filtered homozygous substitutions with CADD < 3. **D** Correlation between inbreeding coefficient (based on runs of homozygosity; F_{ROH}) and Ne. **E** Correlation between heterozygous load and inbreeding coefficient. **F** Correlation between estimated homozygous load and inbreeding coefficient.

Figure 3. Genetic diversity, genetic load, and demographic history of Falconiformes, Psittaciformes, and Columbiformes. A Genome-wide heterozygosity in het x bp^{-1}, **B** inbreeding coefficient, and **C** homozygous load distribution across the orders of the three target species. The inbreeding coefficient (F_{ROH}) was estimated using runs of homozygosity, and homozygous load was based on the ratio of homozygous substitutions with CADD scores above 20 to those with CADD scores below 3. **D** Demographic histories are shown as variation in Ne (effective population size) inferred with PSMC. The falcons had the lowest historical Ne ($5.0x10^4$, harmonic mean for 10 to 100 kya) on average, while the parrots having higher Ne ($7.6x10^4$) and pigeons having the highest Ne ($2.3x10^5$) Thick lines refer to Mauritius species. Only species with chromosomal-level assemblies were included.

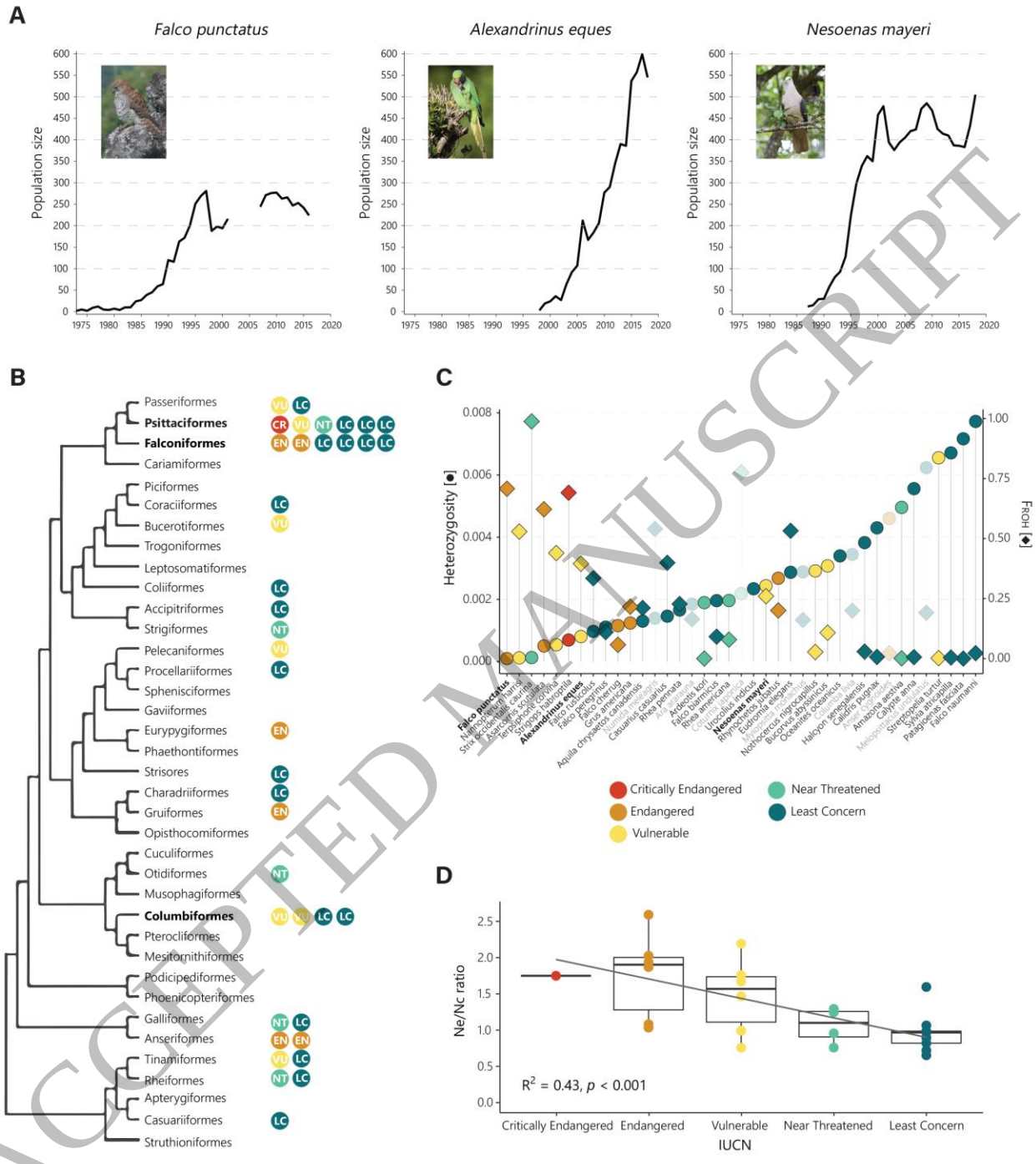

Figure 1
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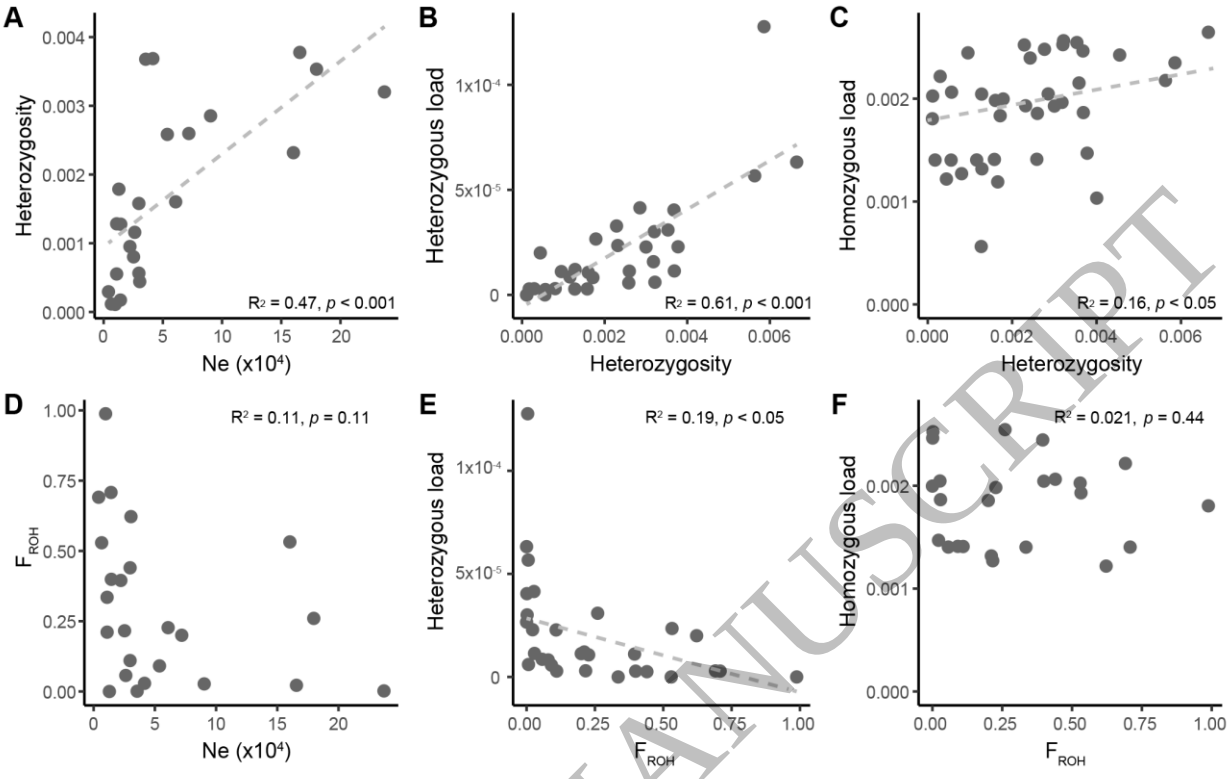

Figure 2
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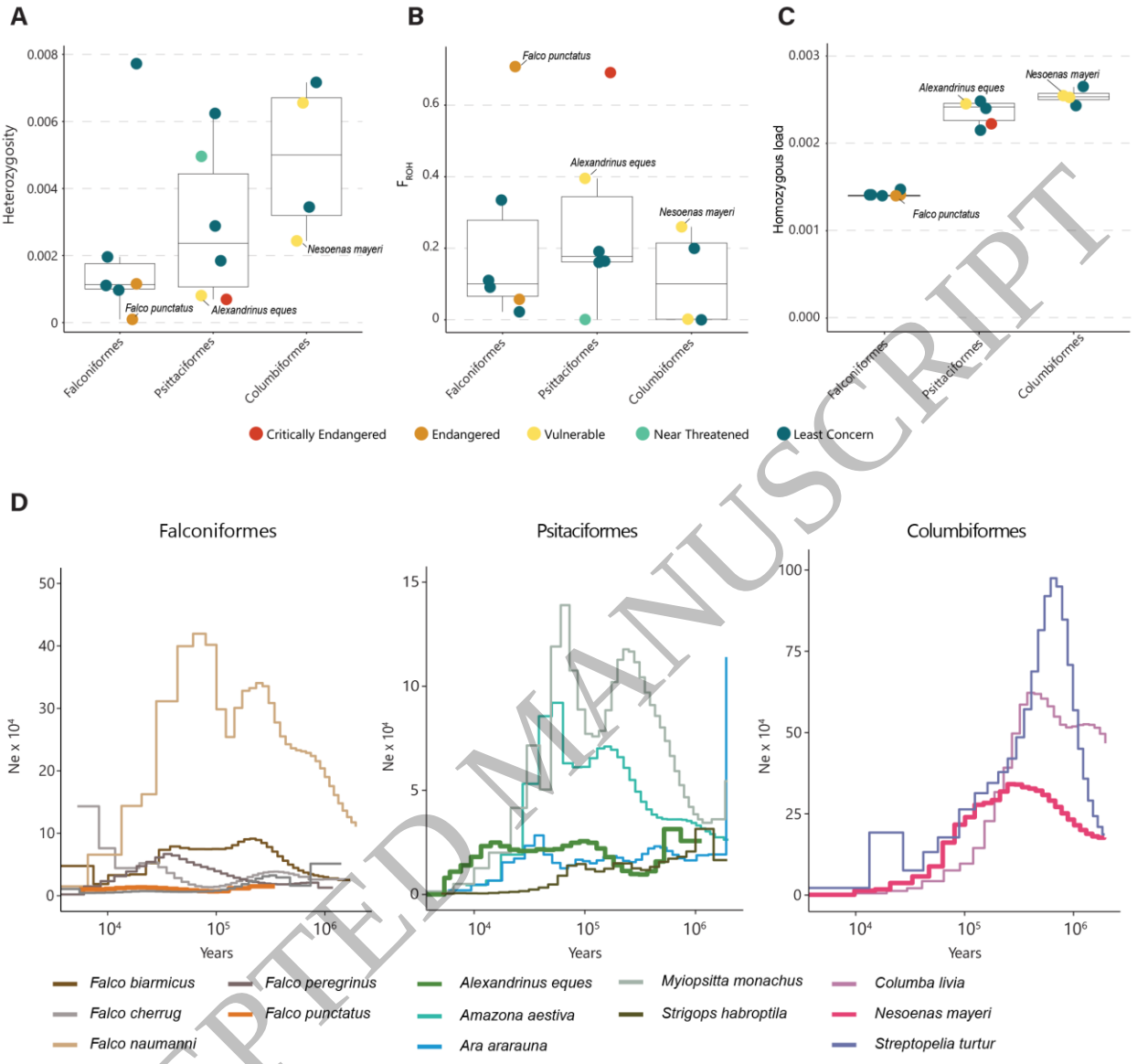

Figure 3
165x154 mm (x DPI)