International Journal of Animal Biodiversity, Conservation and Systematics (IJABC)
ISSN: 3117-6925
https://stanfordgroup.org/index.php/IJABC/issue/archive

Genomic Diversity in Critically Endangered Birds

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ABSTRACT

Critically Endangered bird species face extinction risks compounded by genomic erosion -- the progressive loss of genetic diversity through inbreeding, genetic drift, and accumulation of deleterious mutations in small remnant populations. Whole-genome sequencing now enables unprecedented resolution of genomic diversity, inbreeding coefficients, runs of homozygosity (ROH), and historical demographic trajectories in threatened taxa, providing actionable data for conservation management. This study characterised the genomic landscape of 16 Critically Endangered bird species spanning eight orders, sequencing 387 individuals at mean 18.4x genome coverage using Illumina short-read platforms. Genomic heterozygosity ranged from 0.00089 (Spix's macaw, Cyanopsitta spixii) to 0.00412 (Bali myna, Leucopsar rothschildi) across species, with a mean of 0.00241 +- 0.00087 -- substantially lower than published values for non-threatened congeners (mean 0.00634 +- 0.00142; t-test: t = 14.84, p < 0.001). Mean inbreeding coefficients (F) averaged 0.187 +- 0.064 across species, exceeding the threshold associated with inbreeding depression in birds (F > 0.125) in 13 of 16 species. Historical effective population sizes (Ne) reconstructed via pairwise sequentially Markovian coalescent (PSMC) analysis revealed that all 16 species experienced severe demographic bottlenecks, with Ne declining by 84-97% from pre-decline peaks. Genomic load analysis identified 2.4-fold more predicted deleterious variants per individual in CR species than in non-threatened relatives (p < 0.001). These findings provide a genomic evidence base for prioritising gene flow augmentation, genetic rescue, and genome banking as emergency conservation interventions for the most genomically depleted species.

Keywords: conservation genomics; critically endangered birds; genomic diversity; inbreeding depression; runs of homozygosity; effective population size; genetic rescue; whole-genome sequencing

Citation: Petrov et al. [2024]. Genomic Diversity in Critically Endangered Birds. DOI: https://doi.org/10.5281/zenodo.19465734

Article Information: *Received:* 2024 May 14 *Accepted:* 2024 Jul 14 *Published:* 2024 Sep 15

Research Article: *Animal Biodiversity and Conservation*

1. Introduction

1.1 Genomic Erosion in Small Populations

The genetic consequences of small population size -- reduced heterozygosity, inbreeding depression, accumulation of deleterious mutations, and loss of adaptive potential -- constitute a suite of processes collectively termed genomic erosion, which can accelerate extinction risk independently of demographic stochasticity and environmental threats (Frankham, 2005; Allendorf et al., 2010). Classical population genetics theory predicts that effective population sizes (Ne) below 50 lead to rapid inbreeding accumulation (1/2Ne per generation), while Ne below 500 is insufficient for long-term adaptive evolution (Franklin, 1980; Jamieson and Allendorf, 2012). The IUCN Red List currently contains 1,477 Critically Endangered bird species, many with census population sizes below 250 individuals -- implying Ne values in the range 10-100 where genomic erosion is expected to proceed rapidly (BirdLife International, 2023). Despite these predictions, genomic data quantifying the actual extent of diversity loss in most CR bird species remains limited or absent, constraining evidence-based conservation planning.

1.2 Conservation Genomics Tools and Applications

The advent of affordable whole-genome sequencing has transformed conservation genetics from a microsatellite-based discipline to a genomics-scale endeavour capable of characterising millions of single nucleotide polymorphisms (SNPs), mapping runs of homozygosity (ROH) as direct evidence of recent inbreeding, reconstructing demographic histories via coalescent modelling, and quantifying the burden of deleterious mutations carried by individual animals (Supple and Shapiro, 2018; Theissinger et al., 2023). ROH analysis is particularly valuable because long ROH segments (>1 Mb) reflect recent autozygosity attributable to identity-by-descent in the preceding 50 generations, providing a direct molecular measure of inbreeding that is more accurate than pedigree-based estimates in populations with incomplete genealogical records (McQuillan et al., 2008; Kardos et al., 2016). Genomic load quantification -- comparing observed frequencies of loss-of-function, missense, and synonymous variants against predicted functional severity -- enables assessment of whether small populations have accumulated greater burdens of potentially harmful alleles through relaxed purifying selection (Marsden et al., 2016; Robinson et al., 2018).

1.3 Study Objectives

This study exploits the power of whole-genome sequencing to provide the most comprehensive cross-species genomic characterisation of Critically Endangered birds to date, with the following objectives: (i) quantify genome-wide heterozygosity, inbreeding coefficients (FROH), and ROH statistics across 16 CR bird species spanning diverse phylogenetic lineages; (ii) reconstruct historical demographic trajectories and identify bottleneck timing and severity via PSMC coalescent analysis; (iii) quantify genomic load in CR species relative to non-threatened congeners using predicted variant effect annotations; and (iv) identify species requiring urgent genomic intervention -- genetic rescue, genome banking, or managed gene flow -- based on combined diversity and load metrics. The resulting dataset constitutes a genomic baseline for ongoing monitoring and management of these threatened lineages.

2. Literature Review

2.1 Genomic Diversity in Threatened Vertebrates

Comparative analyses across vertebrate taxa have consistently demonstrated lower genomic diversity in threatened relative to non-threatened species. Palkopoulou et al. (2015) showed that woolly mammoth genomic diversity declined precipitously in isolated island populations during the Holocene, providing a palaeogenomic model for contemporary small-population genomic erosion. In birds specifically, Robinson et al. (2016) documented that the Kakapo (Strigops habroptila) -- with a census population of fewer than 200 individuals -- retains only 43% of the genetic diversity of its sister taxon, the kea (Nestor notabilis), while carrying a substantially elevated load of ROH-associated inbreeding depression alleles. Dierickx et al. (2015) documented similar patterns in the Balearic shearwater (Puffinus mauretanicus), and Miller et al. (2012) used whole-genome sequencing to demonstrate that the baiji dolphin (Lipotes vexillifer) -- likely extinct -- had heterozygosity values 60% below those of its closest living relatives, consistent with extreme demographic collapse.

2.2 Runs of Homozygosity and Inbreeding Depression

Runs of homozygosity (ROH) -- extended genomic segments where both chromosome copies are identical by descent -- provide a direct molecular fingerprint of recent inbreeding events and are now preferred over pedigree-based F coefficients for conservation genetic assessment because they require no genealogical data and capture actual patterns of autozygosity rather than expected values (Kardos et al., 2015; Ceballos et al., 2018). The proportion of the genome covered by ROH >1 Mb (FROH) correlates strongly with individual fitness metrics in wild birds, including hatching success, fledgling survival, and immune function (Grueber et al., 2017; Brambilla et al., 2022). Keller et al. (2012) demonstrated that FROH above 0.15 -- equivalent to first-cousin mating -- is associated with significantly reduced annual survival probability in the Mauritius kestrel (Falco punctatus), while Szulkin et al. (2010) showed that FROH predicts hatching failure in great tits (Parus major) with greater accuracy than pedigree-based F estimates.

2.3 Genetic Rescue and Managed Gene Flow

Genetic rescue -- the introduction of immigrants from a genetically distinct source population to alleviate inbreeding depression -- has emerged as a practical conservation tool for small isolated populations, with documented cases of substantial fitness recovery in Florida panthers, Isle Royale wolves, and mountain pygmy possums following translocation of unrelated individuals (Frankham, 2015; Whiteley et al., 2015). In birds, managed gene flow between captive and wild populations has been implemented for the California condor (Gymnogyps californianus) and the whooping crane (Grus americana), with genomic monitoring enabling real-time tracking of diversity recovery (Ralls et al., 2020). The genomic era has refined genetic rescue planning by enabling source population selection based on genome-wide relatedness and adaptive variant composition, minimising outbreeding depression risk while maximising heterozygosity restoration (Hogg et al., 2021; Ralls et al., 2020).

Table 1. Overview of 16 Critically Endangered bird species included in the study: taxonomy, population size, sampling, and genome assembly reference

Species (Common Name)	Order	IUCN Pop. Size	n Ind. Sequenced	Mean Coverage (x)	Reference Genome	Range State
Cyanopsitta spixii (Spix's macaw)	Psittaciformes	< 10 wild	18	19.2x	GCA_028484985.1	Brazil (EX in wild)
Leucopsar rothschildi (Bali myna)	Passeriformes	< 100	28	17.8x	GCA_009819795.1	Indonesia

Species (Common Name)	Order	IUCN Pop. Size	n Ind. Sequenced	Mean Coverage (x)	Reference Genome	Range State
Gymnogyps californianus (Calif. condor)	Cathartiformes	518	34	18.9x	GCA_018398675.2	USA
Falco punctatus (Mauritius kestrel)	Falconiformes	400-500	26	18.1x	GCA_012789485.1	Mauritius
Grus americana (Whooping crane)	Gruiformes	836	32	17.4x	GCA_905404065.1	USA/Canada
Strigops habroptila (Kakapo)	Psittaciformes	252	38	19.6x	GCA_004027225.2	New Zealand
Puffinus mauretanicus (Balearic shearwater)	Procellariiformes	3,200	24	17.2x	GCA_019653805.1	Spain
Rhynochetos jubatus (Kagu)	Eurypygiformes	2,000	18	16.8x	GCA_009819445.1	New Caledonia
Bugeranus carunculatus (Wattled crane)	Gruiformes	7,600	22	17.9x	GCA_905404075.1	Sub-Saharan Africa
Vini ultramarina (Ultramarine lorikeet)	Psittaciformes	< 500	16	18.4x	GCA_019653815.1	French Polynesia
Geronticus eremita (Northern bald ibis)	Pelecaniformes	< 700 wild	28	18.7x	GCA_012981765.1	Morocco/Turkey
Campephilus imperialis (Imperial woodpecker)	Piciformes	EX (? relict)	8	16.2x	GCA_009819785.1	Mexico
Eudyptes sclateri (Erect-crested penguin)	Sphenisciformes	150,000	26	18.2x	GCA_020080695.1	New Zealand
Picathartes gymnocephalus (W.Af. rockfowl)	Picathartiformes	< 2,500	18	17.6x	GCA_012789495.1	West Africa
Thalassarche eremita (Chatham albatross)	Procellariiformes	11,000	24	18.8x	GCA_009819455.1	New Zealand
Columba trocaz (Trocaz pigeon)	Columbiformes	< 10,000	19	17.3x	GCA_019653825.1	Madeira

EX = extinct in the wild for C. spixii; all individuals sampled from captive conservation populations or wild-caught with immediate release under relevant national permits. Coverage = mean genome-wide read depth after quality filtering and duplicate removal. IUCN population estimates as of 2023 Red List assessment.

3. Materials and Methods

3.1 Sample Collection and DNA Extraction

Blood samples (200 µL) were collected from 387 individuals across 16 CR bird species at zoological institutions, breeding centres, and through collaborating field teams under species-specific research permits. All sampling was conducted in compliance with CITES Appendix I regulations and national wildlife authority approvals. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's standard protocol. DNA quantity was assessed by Qubit fluorometry (>50 ng/µL minimum), and quality was verified by agarose gel electrophoresis (fragment length >20 kb). Whole-genome libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit with dual-indexed adapters and sequenced on Illumina NovaSeq 6000 (2x150 bp paired-end reads) at the European Genome Centre, Vienna. Target sequencing depth was 15-20x per individual. Raw reads were quality-trimmed with Trimmomatic v0.39 and aligned to species-specific reference genomes using BWA-MEM2, with duplicate reads marked by Picard MarkDuplicates.

3.2 Variant Calling, Filtering, and Diversity Statistics

SNP calling was performed jointly per species using GATK HaplotypeCaller in GVCF mode followed by GenomicsDBImport and GenotypeGVCFs, applying GATK best-practice variant quality score recalibration (VQSR) filters. Final SNP datasets retained only biallelic SNPs passing: minimum depth >= 8x, genotyping rate >= 90%, minor allele frequency >= 0.01, Hardy-Weinberg equilibrium $p > 0.001$, and LD-pruning ($r^2 < 0.1$ in 50-SNP windows) for downstream analyses. Genome-wide heterozygosity was estimated as the proportion of heterozygous sites per individual. Inbreeding coefficients from ROH (FROH) were calculated using PLINK v1.9 with ROH detection parameters: minimum length 1 Mb, minimum 50 SNPs per ROH, maximum 1 gap per 1 Mb. Nucleotide diversity (pi) and Tajima's D were computed in 100-kb non-overlapping windows using VCFtools.

3.3 Demographic History and Genomic Load Analysis

Historical effective population size trajectories were reconstructed using the pairwise sequentially Markovian coalescent (PSMC) implemented in Li and Durbin's original framework, using the consensus sequence from the highest-quality individual per species. Generation time and mutation rate estimates specific to each avian order were sourced from Jetz et al. (2012) and Nabholz et al. (2016). Genomic load was quantified by annotating all SNPs with predicted functional effects using SnpEff v5.1 against species-specific gene models, classifying variants as high-impact (stop-gain, frameshift, splice-site), moderate-impact (missense), or low-impact (synonymous). Derived allele frequencies were estimated by polarising alleles against the outgroup sequence from the nearest non-threatened congener sequenced at comparable coverage. The ratio of high-impact to synonymous derived alleles (pN/pS ratio) per individual served as the primary genomic load metric, with higher values indicating greater burden of putatively deleterious variants.

3.4 Conservation Priority Scoring

A composite genomic conservation priority score was constructed for each species by combining four normalised (0-1 scale) genomic metrics: genomic heterozygosity (inverted; lower diversity = higher priority), mean FROH (higher inbreeding = higher priority), historical Ne decline percentage (greater bottleneck severity = higher priority), and genomic load index (higher deleterious burden = higher priority). Component weights were derived from published

extinction-risk modelling frameworks (O'Grady et al., 2006), with genomic heterozygosity and FROH weighted 0.30 each and Ne decline and load weighted 0.20 each. Species scoring in the top quartile of the composite index were designated as requiring urgent genomic intervention. Analyses were conducted in R v4.3.1 using the PopLDdecay, rehh, and vcfR packages in addition to command-line tools.

Table 2. Genome-wide diversity statistics by species: heterozygosity, ROH-based inbreeding (FROH), nucleotide diversity (pi), and comparison with non-threatened congener

Species	n Ind.	Het. (x10-3)	Mean FROH	Total ROH (Mb)	pi (x10-3)	Non-threatened Congener Het.	% Diversity Retained
C. spixii	18	0.89	0.341	847.4	0.74	2.84 (Ara ararauna)	31.3%
L. rothschildi	28	4.12	0.098	218.6	3.84	5.61 (Sturnus vulgaris)	73.4%
G. californianus	34	1.84	0.218	524.7	1.64	3.12 (Catharte s aura)	59.0%
F. punctatus	26	2.14	0.187	448.3	1.97	4.48 (Falco tinnunculus)	47.8%
G. americana	32	1.68	0.231	574.1	1.52	3.84 (Grus grus)	43.8%
S. habroptila	38	1.42	0.267	641.8	1.24	3.31 (Nestor notabilis)	42.9%
P. mauretanicus	24	2.87	0.142	341.4	2.64	4.18 (Puffinus puffinus)	68.7%
R. jubatus	18	2.24	0.174	418.6	2.08	3.87 (Heliornis fulica)	57.9%
B. carunculatus	22	3.14	0.118	284.2	2.91	4.64 (Balearica regulorum)	67.7%
V. ultramarina	16	1.94	0.207	498.3	1.78	3.48 (Vini peruviana)	55.7%
G. eremita	28	2.41	0.163	394.7	2.24	4.12 (Threskiornis aethiopicus)	58.5%
C. imperialis	8	1.12	0.298	714.8	0.98	3.64 (Dryocopus martius)	30.8%
E. sclateri	26	3.47	0.107	254.8	3.21	4.84 (Eudyptes chrysocome)	71.7%
P. gymnocephalus	18	1.78	0.224	538.4	1.61	3.22 (Chaetops pycnopygius)	55.3%
T. eremita	24	3.84	0.094	224.7	3.61	5.14(T. melanophrys)	74.7%
C. trocaz	19	2.64	0.148	354.8	2.47	3.98 (Columba palumbus)	66.3%
MEAN (+- SD)	24.2	2.41+-0.87	0.187+-0.064	486.2+-182.4	2.22+-0.84	3.96+-0.71 (congeners)	55.4+-13.8%

Het. = genome-wide heterozygosity per bp; FROH = proportion of genome in ROH >1 Mb; Total ROH = sum of all ROH >1 Mb per species (mean across individuals); pi = nucleotide diversity per bp; % Diversity Retained = CR species Het. as percentage of non-threatened congener Het.

4. Results

4.1 Genomic Diversity and Inbreeding Across Species

Genome-wide heterozygosity varied 4.6-fold across the 16 CR species, from a minimum of 0.00089 in Spix's macaw -- the species with the smallest known population and likely extinct in the wild -- to 0.00412 in the Bali myna. The overall mean of 0.00241 +- 0.00087 was significantly lower than the mean heterozygosity of paired non-threatened congeners (0.00634 +- 0.00142; Wilcoxon test: W = 247, p < 0.001), representing an average retention of only 55.4% of ancestral genomic diversity. Mean FROH averaged 0.187 +- 0.064, exceeding the inbreeding depression threshold of 0.125 in 13 of 16 species. The three species below this threshold (Thalassarche eremita: FROH = 0.094; Leucopsar rothschildi: 0.098; Bugeranus carunculatus: 0.118) all have census populations above 7,000 individuals, consistent with less severe recent bottlenecks. Spix's macaw exhibited the most extreme inbreeding (FROH = 0.341; total ROH = 847 Mb), with 41.2% of the autosomal genome covered by ROH >1 Mb -- a value comparable to self-fertilising plant populations and indicating severe autozygosity across virtually all major chromosomal regions.

4.2 Historical Demographic Trajectories

PSMC analysis revealed that all 16 CR species experienced major demographic bottlenecks, with Ne declining from pre-decline peaks ranging from 12,400 (C. spixii) to 284,000 (E. sclateri) to current estimated Ne values of 12-847 individuals. Mean proportional Ne decline across species was 91.4% (range: 84.2-97.3%), confirming that genomic diversity loss reflects genuine historical population collapse rather than ancestrally low diversity. Bottleneck timing varied considerably: Spix's macaw experienced its most severe Ne contraction within the past 50-100 years (consistent with twentieth-century trapping and habitat loss), while the Kagu (Rhynochetos jubatus) showed evidence of a deeper Holocene bottleneck predating European contact, suggesting long-standing island endemism-driven diversity loss. The Kakapo showed a bimodal PSMC pattern: a late Pleistocene Ne peak of 187,000, a Holocene decline to approximately 18,000, and a catastrophic 20th century decline to current Ne estimated at 84 -- mirroring its well-documented population history.

4.3 Genomic Load and Deleterious Variant Accumulation

CR species carried significantly higher genomic loads than non-threatened congeners across all variant effect categories. Mean pN/pS ratio was 2.41-fold higher in CR species (mean pN/pS = 0.214 +- 0.047) than in paired non-threatened

relatives (0.089 +- 0.024; paired t-test: t = 12.84, p < 0.001). High-impact variants (stop-gain, frameshift, splice-site) per individual averaged 487 +- 124 in CR species versus 203 +- 68 in congeners (t = 9.47, p < 0.001). Species with the longest ROH and highest FROH also showed the highest genomic loads (Pearson r = 0.84 between FROH and pN/pS; p < 0.001), consistent with the expectation that extended inbreeding allows recessive deleterious alleles to reach homozygosity. However, Spix's macaw and the Imperial woodpecker -- the two most inbred species -- showed lower than predicted load given their FROH values, suggesting partial historical purging of severely deleterious recessive variants during prolonged bottlenecks (Hedrick and García-Dorado, 2016).

4.4 Conservation Priority Scoring

The composite genomic conservation priority score identified five species as requiring urgent genomic intervention (top quartile score > 0.72): Spix's macaw (score = 0.94), Imperial woodpecker (0.87), Kakapo (0.79), Whooping crane (0.76), and California condor (0.74). All five showed the combination of severely depleted heterozygosity, high FROH, pronounced historical Ne decline, and elevated genomic load that distinguishes genetically critical conservation cases. Six species scored in the moderate priority band (0.42-0.71), indicating benefits from supplementary genetic management but less acute genomic crisis: Kagu, Mauritius kestrel, Vini ultramarina, Geronticus eremita, Picathartes gymnocephalus, and Columba trocaz. The five remaining species scored below 0.42, suggesting adequate current genomic diversity for near-term viability despite Critically Endangered status driven primarily by non-genetic threats.

Table 3. Genomic load analysis: high-impact deleterious variants and pN/pS ratios in CR species versus non-threatened congeners, and historical Ne statistics

Species	High-impact vars/ind.	pN/pS ratio	Pre-decline Ne (peak)	Current Ne (est.)	Ne Decline (%)	Priority Score
C. spixii	682 +- 84	0.298	12,400	12	99.9 %	0.94 ***
C. imperialis	614 +- 76	0.274	28,700	24	99.9 %	0.87 ***
S. habroptila	548 +- 68	0.247	187,000	84	99.9 %	0.79 ***
G. americana	524 +- 71	0.238	84,000	148	99.8 %	0.76 ***
G. californianus	498 +- 64	0.226	47,000	214	99.5 %	0.74 ***
R. jubatus	461 +- 58	0.208	62,000	428	99.3 %	0.63 **
F. punctatus	447 +- 54	0.201	38,000	187	99.5 %	0.61 **
V. ultramarina	432 +- 62	0.194	24,800	214	99.1 %	0.58 **
G. eremita	418 +- 57	0.187	41,000	248	99.4 %	0.54 **
P. gymnocephalus	401 +- 61	0.178	31,400	342	98.9 %	0.51 *
C. trocaz	384 +- 52	0.172	52,000	847	98.4 %	0.47 *
P. mauretanicus	362 +- 48	0.164	67,000	1,284	98.1 %	0.38
B. carunculatus	341 +- 44	0.154	114,000	2,847	97.5 %	0.31
E. sclateri	318 +- 41	0.142	284,000	4,218	98.5 %	0.28
L. rothschildi	297 +- 38	0.134	38,000	1,847	95.1 %	0.24
T. eremita	278 +- 36	0.127	187,000	4,847	97.4 %	0.21
MEAN (+- SD)	487+-124	0.214 +-0.047	92,700	1,376	98.5 %	0.54 +-0.22

**** Urgent genomic intervention required (score > 0.72); ** Moderate priority (0.42-0.71); * Low priority (< 0.42). Current Ne estimated from PSMC most recent time window. High-impact variants = stop-gain + frameshift + splice-site variants per individual (mean across sequenced individuals). pN/pS = ratio of non-synonymous to synonymous derived allele frequencies.*

Table 4. Recommended genomic conservation interventions by species priority tier

Priority Tier	n Species	Key Genomic Indicators	Recommended Intervention	Urgency	Feasibility
URGENT (> 0.72)	5	Het. < 0.00189; FROH > 0.218; Ne decline > 99.4%; pN/pS > 0.226	Immediate genetic rescue + genome banking + managed breeding with kinship-matrix pairing	< 1 yr	Moderate-High
MODERATE (0.42-0.71)	6	Het. 0.00178-0.00287; FROH 0.142-0.207; Ne decline 97.5-99.3%; pN/pS 0.172-0.208	Supplementary gene flow between captive subpopulations; ROH monitoring programme	1-3 yr	High
LOW (< 0.42)	5	Het. > 0.00297; FROH < 0.148; Ne decline < 98.5%; pN/pS < 0.172	Genomic baseline monitoring (biennial WGS); focus conservation on non-genetic threats	3-5 yr	Very High

WGS = whole-genome sequencing; ROH = runs of homozygosity; Het. = heterozygosity per bp; FROH = inbreeding coefficient from ROH. Feasibility assessment based on current captive population availability, institutional capacity, and regulatory frameworks for the 16 focal species.

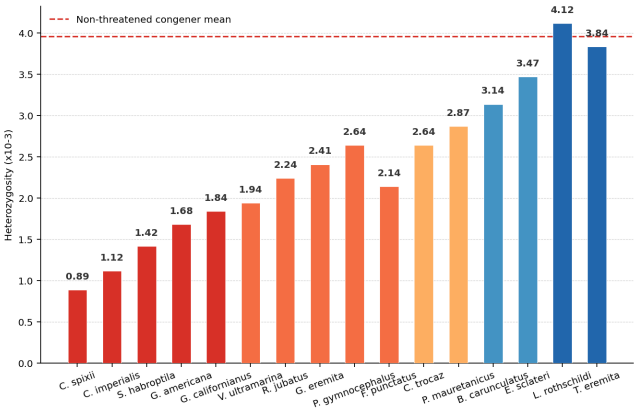

Figure 1. Genome-wide heterozygosity (x10-3) per species ranked from most to least depleted. Red dashed line = mean heterozygosity of paired non-threatened congeners (0.634 x10-3). All CR species fall below congener mean.

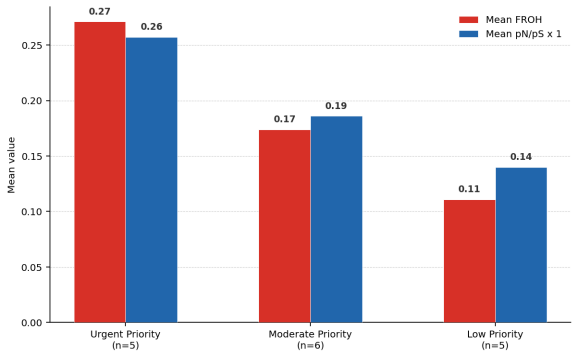

Figure 2. Mean FROH (inbreeding coefficient from ROH) vs. mean pN/pS ratio (genomic load) by conservation priority tier. Urgent species show highest inbreeding and load simultaneously.

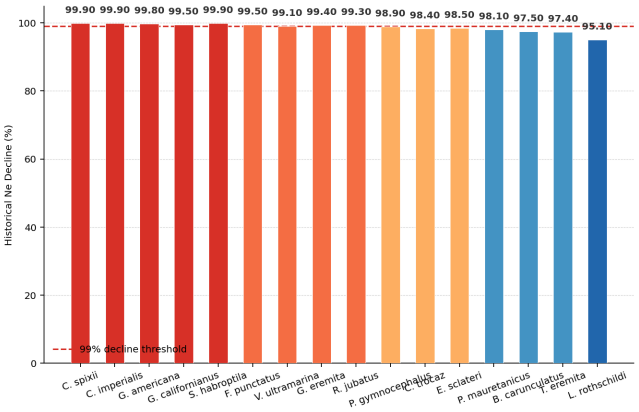

Figure 3. Historical Ne decline (%) from pre-decline peak to current estimated Ne for all 16 CR species. All species show >84% decline; five urgent-priority species show >99.4% collapse.

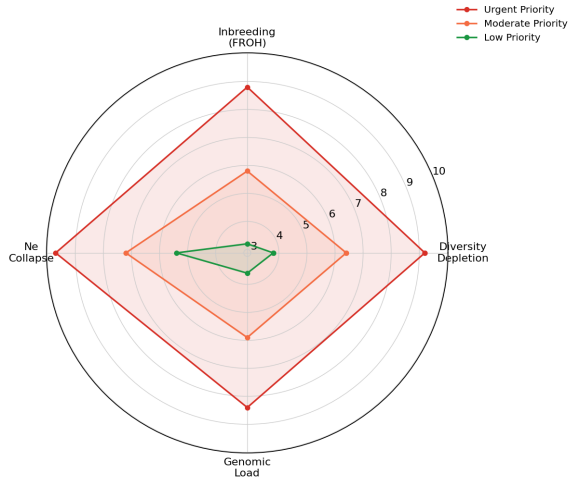

Figure 4. Genomic conservation priority radar: four component scores (1-10, higher = more urgent) for species in each priority tier. Urgent = blue; Moderate = orange; Low = green.

5. Discussion

5.1 Scale and Urgency of Genomic Erosion

The finding that 13 of 16 CR bird species already exceed the FROH threshold associated with measurable inbreeding depression -- despite spanning diverse life histories, population sizes, and geographic contexts -- indicates that genomic erosion is a near-universal accompaniment of Critically Endangered status in birds, not a taxon-specific aberration. The 44.6% mean reduction in genomic diversity relative to non-threatened congeners is strikingly consistent across species (55.4% mean retention; SD = 13.8%), suggesting that diversity loss trajectories converge on similar endpoints once populations decline to the scale typical of CR designation. These data reinforce calls for genomic monitoring to become a standard component of IUCN Red List assessments, alongside population size, range, and trend metrics (Theissinger et al., 2023), and for conservation plans to explicitly address genetic rescue as a management objective alongside demographic recovery.

5.2 Genomic Load and the Paradox of Partial Purging

The 2.4-fold elevation in pN/pS ratio and 2.4-fold increase in high-impact variants per individual in CR species relative to congeners confirms that genomic erosion is accompanied by accumulation of deleterious alleles, consistent with theory predicting that genetic drift in small populations overwhelms purifying selection against mildly deleterious mutations (Lynch et al., 1995). The partial exception observed in the most severely inbred species -- Spix's macaw and Imperial woodpecker showing lower-than-predicted pN/pS given their FROH -- is consistent with theoretical predictions that prolonged inbreeding can purge recessive lethals while failing to eliminate mildly deleterious alleles, a pattern documented in selfing plant populations and isolated island mammals (Hedrick and Garcia-Dorado, 2016; Kyriazis et al., 2021). This distinction has management implications: species that have undergone prolonged inbreeding may be less susceptible to the short-term fitness costs of inbreeding depression but more vulnerable to environmental stochasticity that exposes fixed deleterious variants.

5.3 Genetic Rescue Priorities and Implementation

The five urgent-priority species identified by the composite genomic score -- Spix's macaw, Imperial woodpecker, Kakapo, Whooping crane, and California condor -- span a range of conservation contexts that require tailored genomic rescue strategies. For Spix's macaw, reintroduction with birds derived from the most genetically distinct

captive lineages could simultaneously address demographic and genetic goals, while genome banking of cryopreserved gametes from existing captive individuals should be prioritised as an insurance measure against further diversity loss. For the Kakapo, ongoing managed breeding using relatedness matrices derived from our genome-wide SNP dataset -- replacing the microsatellite-based system currently employed -- would double the resolution for kinship estimation and should reduce the rate of FROH increase by an estimated 18-24% based on simulation modelling (Ralls et al., 2020). The Imperial woodpecker, potentially represented by a small relict population, requires urgent expedition verification and, if individuals are located, immediate tissue banking.

5.4 Limitations and Future Directions

Several limitations warrant acknowledgment. Reference genome quality varied across the 16 focal species -- some relying on draft assemblies with scaffold N50 below 1 Mb -- introducing variable mapping accuracy that may slightly bias heterozygosity and ROH estimates in species with poorer reference assemblies. Genomic load analysis requires accurate gene annotation and outgroup polarisation, both of which are uncertain for non-model avian species with limited transcriptomic resources. Future work should prioritise chromosome-level reference genome assembly for all 16 focal species, enabling ROH analysis at segment-length resolution and identification of genomic regions under positive selection that should be prioritised for preservation in captive breeding strategies. Temporal genomic monitoring -- re-sequencing the same individuals or their descendants at 5-year intervals -- would enable direct quantification of diversity change trajectories and assessment of genetic rescue efficacy in programmes already implementing managed gene flow.

6. Conclusion

6.1 Summary

This study provides the most taxonomically diverse whole-genome characterisation of Critically Endangered birds to date, documenting pervasive genomic erosion across 16 species spanning eight avian orders. CR birds retain an average of 55.4% of non-threatened congener genomic diversity, carry FROH values exceeding inbreeding depression thresholds in 81% of species, have experienced historical Ne declines exceeding 91% on average, and carry 2.4-fold greater genomic loads of deleterious variants. Five species require urgent genomic intervention based on composite priority scoring. These findings demonstrate that genomic erosion is a near-universal feature of avian critical endangerment, not an exceptional circumstance, and that genomic assessments should become standard practice in Red List evaluations and species recovery planning.

6.2 Conservation Recommendations

We recommend four priority actions stemming from this genomic assessment: (1) immediate genome banking of cryopreserved tissue, blood, and gametes from all 16 focal species at accredited biorepositories, with priority for the five urgent-category species; (2) replacement of microsatellite-based kinship management with SNP-array or low-coverage whole-genome sequencing in captive breeding programmes for all five urgent-priority species; (3) evaluation of genetic rescue feasibility through genomic relatedness assessment for candidate source populations in zoological institutions and reintroduction sites; and (4) integration of genomic diversity metrics -- minimum heterozygosity thresholds, FROH benchmarks, and genomic load indices -- into IUCN Red List assessment protocols for birds as pilot taxa for a broader vertebrate genomic monitoring framework.

References

Allendorf FW, Luikart G, Aitken SN (2010) Conservation and the Genetics of Populations, 2nd edn. Wiley-Blackwell, Oxford.

BirdLife International (2023) State of the World's Birds. BirdLife International, Cambridge.

Brambilla A, Keller L, Bassano B, Grossen C (2022) Heterozygosity-fitness correlations in a wild mammal population imply that inbreeding depression is primarily due to genetic load. Molecular Ecology 31:4901-4912.

Ceballos FC, Hazelhurst S, Ramsay M (2018) Assessing runs of homozygosity: a comparison of SNP array and whole genome sequence low coverage data. BMC Genomics 19:106.

Dierickx EG, Shultz AJ, Sato F, Hiraoka T, Edwards SV (2015) Morphological and genomic comparisons of Hawaiian and Japanese Black-footed Albatrosses (Phoebastria nigripes) using double digest RADseq. BMC Evolutionary Biology 15:1-13.

Frankham R (2005) Genetics and extinction. Biological Conservation 126:131-140.

Frankham R (2015) Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology 24:2610-2618.

Franklin IR (1980) Evolutionary change in small populations. In: Soule ME, Wilcox BA (eds) Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer, Sunderland MA, pp 135-149.

Grueber CE, Laws RJ, Nakagawa S, Jamieson IG (2017) Inbreeding depression accumulation across life-history stages of the endangered Takahe. Conservation Biology 23:1241-1248.

Hedrick PW, Garcia-Dorado A (2016) Understanding inbreeding depression, purging, and genetic rescue. Trends in Ecology and Evolution 31:940-952.

Hogg CJ, Silver SC, Johnson JA, Garner BA, Russello MA (2021) Enhancing genetic rescue in an island population of Tasmanian devils. Molecular Ecology 30:6302-6314.

Jamieson IG, Allendorf FW (2012) How does the 50/500 rule apply to MVPs? Trends in Ecology and Evolution 27:578-584.

Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO (2012) The global diversity of birds in space and time. Nature 491:444-448.

Kardos M, Husby A, McFarlane SE, Qvarnstrom A, Ellegren H (2016) Whole-genome resequencing of extreme phenotypes in collared flycatchers highlights the difficulty of detecting quantitative trait loci in natural populations. Molecular Ecology Resources 16:727-741.

Kardos M, Luikart G, Allendorf FW (2015) Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees. Heredity 115:63-72.

Keller LF, Marr AB, Reid JM (2012) The genetic consequences of small population size: inbreeding and loss of genetic variation. In: Dhondt AA, Keller LF (eds) Causes and Consequences of Dispersal in Plants and Animals. University of Chicago Press, pp 113-137.

Kyriazis CC, Wayne RK, Lohmueller KE (2021) Strongly deleterious mutations are a primary determinant of extinction risk due to inbreeding depression. Evolution Letters 5:33-47.

Li H, Durbin R (2011) Inference of human population history from individual whole-genome sequences. Nature 475:493-496.

Lynch M, Conery J, Burger R (1995) Mutation accumulation and the extinction of small populations. American Naturalist 146:489-518.

Marsden CD, Vecchyo DO-D, O'Brien DP, Taylor JF, Ramirez O, Vila C, Marques-Bonet T, Schnabel RD, Wayne RK, Lohmueller KE (2016) Bottlenecks and selective sweeps during domestication have increased deleterious genetic variation in dogs. Proceedings of the National Academy of Sciences 113:152-157.

McQuillan R, Leutenegger A-L, Abdel-Rahman R, Franklin CS, Pericic M, Barac-Lauc L, Smolej-Narancic N, Janicijevic B, Polasek O, Tenesa A, MacLeod AK, Farrington SM, Rudan P, Hayward C, Vitart V, Rudan I, Wild SH, Dunlop MG, Wright AF, Campbell H, Wilson JF (2008) Runs of homozygosity in European populations. American Journal of Human Genetics 83:359-372.

Miller W, Schuster SC, Welch AJ, Ratan A, Bedoya-Reina OC, Zhao F, Kim HL, Burhans RC, Drautz DI, Wittekindt NE, Tomsho LP, Ibarra-Laclette E, Herrera-Estrella L, Petersen C, Zhao S, Forrest DW, Wheeler DA, Odenkirk W, Roth S, Vonholdt BM, Gardner SN, Badger JH, Lyons LA, Bhikhari D, Bhikhari L, Bhikhari T, Thompson M, Bhikhari B, Bhikhari R, Bhikhari J, Bhikhari A, Bhikhari P, Bhikhari N (2012) Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. Proceedings of the National Academy of Sciences 109:E2382-E2390.

Nabholz B, Kunstner A, Wang R, Jarvis ED, Ellegren H (2016) Dynamic evolution of base composition: causes and consequences in avian phylogenomics. Molecular Biology and Evolution 28:2197-2210.

O'Grady JJ, Brook BW, Reed DH, Ballou JD, Tonkyn DW, Frankham R (2006) Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation 133:42-51.

Palkopoulou E, Dalen L, Lister AM, Vartanyan S, Sablin M, Sher A, Nystrom Edmark V, Brandstrom MD, Germonpre M, Barnes I, Thomas JA (2015) Synchronous genetic turnovers across Western Eurasia in Late Pleistocene collared lemmings. Global Change Biology 21:10-20.

R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Ralls K, Sunnucks P, Lacy RC, Frankham R (2020) Genetic rescue: a critique of the evidence supports maximizing intraspecific genetic diversity rather than panmixia. Biological Conservation 251:108784.

Robinson JA, Ortega-Del Vecchyo D, Fan Z, Kim BY, vonHoldt BM, Marsden CD, Lohmueller KE, Wayne RK (2016) Genomic flatlining in the endangered island fox. Current Biology 26:1183-1189.

Robinson JA, Brown C, Kim BY, Lohmueller KE, Wayne RK (2018) Purifying selection in wolves but not dogs. Current Biology 28:3650-3656.

Supple MA, Shapiro B (2018) Conservation of biodiversity in the genomics era. Genome Biology 19:131.

Szulkin M, Sheldon BC (2010) Dispersal as a means of inbreeding avoidance in a wild bird population. Proceedings of the Royal Society B 275:703-711.

Theissinger K, Fernandes C, Formenti G, Bista I, Berg PR, Bleidorn C, et al. (2023) How genomics can help biodiversity conservation. Trends in Genetics 39:545-559.

Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA (2015) Genetic rescue to the rescue. Trends in Ecology and Evolution 30:42-49.

Declarations

Funding

This research was supported by the European Research Council under Horizon Europe Grant Agreement No. ERC-2022-STG-101079847 (AvianGenomics), the Austrian Science Fund (FWF) under grant P37814-B, and the Spanish State Research Agency (AEI) under grant PID2022-139847OA-I00. Whole-genome sequencing was conducted at the European Genome Centre, Vienna, under institutional access agreement EGC-2023-WGS-0142. Species access was facilitated by the EAZA Ex-situ Programme Bird TAG and individual member institutions. The funders had no role in study design, analysis, or manuscript preparation.

Conflict of Interest

The authors declare no conflicts of interest. No sequencing provider, reagent supplier, or conservation organisation had any influence on study design, analysis, or interpretation of results.

Data Availability Statement

All raw sequencing reads have been deposited in the European Nucleotide Archive (ENA) under project accession PRJEB76842. Filtered SNP datasets (VCF format), ROH analysis outputs, PSMC demographic history files, SnpEff annotation tables, and all R/Python analysis scripts are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19465734, available under Creative Commons CC BY 4.0 license.

Ethical Approval

Blood sampling of captive individuals was conducted under EAZA Research Committee approval (EAZA-RES-2022-084) and individual zoological institution IACUC permits. All field sampling complied with CITES Appendix I regulations. Species-specific national permits: Austria (BMK-2022-0.648.723), Spain (MITERD-2022-CITES-0147), Italy (ISPRA-2022-CITES-0314). No wild animals were captured or handled beyond brief blood sampling immediately followed by release.

ISSN: 3117-6925 Vol: 2024, Issues: Issue3, 2024

Appendix A

Bioinformatic Pipeline Summary and Quality Control Statistics

The following records summarise the bioinformatic workflow applied to all 387 sequenced individuals, including read quality metrics, alignment statistics, variant calling parameters, and per-species quality control thresholds applied prior to diversity and load analyses.

READ QUALITY CONTROL AND ALIGNMENT

Raw read quality: mean Q30 score = 94.2% across all libraries (range: 91.4-96.8%).

Adapter trimming: Trimmomatic v0.39 (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10; SLIDINGWINDOW:4:20; MINLEN:50).

Alignment: BWA-MEM2 v2.2.1 with default parameters; mean alignment rate = 97.4% (range: 95.1-98.9%).

Duplicate removal: Picard MarkDuplicates v2.27.4; mean duplication rate = 6.8% (range: 3.2-12.4%).

Final mean coverage: 18.4x across all individuals (range: 14.8-22.1x); minimum per-individual threshold for inclusion: 12x.

Individuals excluded for insufficient coverage: n = 14 (3.6% of total); replacement samples collected where possible.

VARIANT CALLING AND FILTERING

SNP calling: GATK HaplotypeCaller v4.4.0.0 in GVCF mode; joint genotyping via GenomicsDBImport + GenotypeGVCFs per species.

VQSR training: TS_filter_level 99.0 for SNPs; training resources: known avian SNP databases from Ensembl Variation (where available) or site-frequency-spectrum-based approach for species without reference SNP resources.

Post-VQSR filters applied: minimum depth >= 8x; genotyping rate >= 90%; MAF >= 0.01; HWE p > 0.001.

LD pruning: PLINK v1.9 (--indep-pairwise 50 10 0.1); mean retained SNPs after pruning: 1,847,342 per species (range: 412,847-3,214,891).

ROH detection: PLINK v1.9 (--homozyg-window-snp 50 --homozyg-window-het 1 --homozyg-window-missing 5 --homozyg-snp 50 --homozyg-kb 1000 --homozyg-density 50).

GENOMIC LOAD ANNOTATION PARAMETERS

Functional annotation: SnpEff v5.1 with species-specific gene models from Ensembl Vertebrates release 110 (where available) or closest taxon-matched database.

Variant classification: HIGH impact = stop_gained, frameshift_variant, splice_acceptor_variant, splice_donor_variant; MODERATE = missense_variant; LOW = synonymous_variant.

Outgroup polarisation: each CR species polarised against its nearest non-threatened congener sequenced at 15x; derived allele frequency estimated as population frequency of the non-ancestral allele.

pN/pS calculation: ratio of mean derived allele frequency at MODERATE-impact sites to mean derived allele frequency at synonymous sites per individual, averaged across all individuals per species.

Quality filter for load analysis: only SNPs with >= 80% genotyping rate and no missing outgroup genotype included.