Genomic consequences of isolation and inbreeding in an island dingo population

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ABSTRACT

Dingoes come from an ancient canid lineage that originated in East Asia around 8000-11,000 years BP. As Australia's largest terrestrial predator, dingoes play an important ecological role. A small population exists on a world heritage listed offshore island, K'gari (formerly Fraser Island). While dingoes on K'gari are protected, dingoes on the mainland are only protected in certain regions, and, because of its controversial status in Australia, lethal control is commonly used for management. Concern regarding the persistence of dingoes on K'gari has risen due to their low genetic diversity and elevated inbreeding levels. However, whole-genome sequencing data is lacking from this population. Here, we include five new whole-genome sequences of K'gari dingoes. We analyze a total of

18 whole genome sequences of dingoes sampled from mainland Australia and K'gari to assess the genomic consequences of their demographic histories. Long (>1 Mb) runs of homozygosity (ROH) — indicators of inbreeding — are elevated in all sampled dingoes. However, K'gari dingoes showed significantly higher levels of very long ROH (>5 Mb), providing genomic evidence for small population size, isolation, inbreeding, and a strong founder effect. Our results suggest that, despite current levels of inbreeding, the K'gari population is purging strongly deleterious mutations, which, in the absence of further reductions in population size, may facilitate the persistence of small populations despite low genetic diversity and isolation. However, there is little to no purging of mildly deleterious alleles, which may have important long-term consequences, and should be considered by conservation and management programs.

INTRODUCTION

The taxonomic classification of the dingo within the *Canis* genus, as well as its identity are surrounded by controversy. Debate on the most appropriate nomenclature and taxonomic classification centers on whether the dingo should be classified as *Canis familiaris* (Jackson et al. 2017; Jackson et al. 2019) or *Canis dingo* (Crowther et al. 2014; Smith et al. 2019), depending on how the species concept is defined and employed. The different lines of thought and opinions on the dingo's evolutionary history, its relationship with wolves and domestic dogs, its domestication status, and its arrival to Australia has made it difficult to agree on how to manage and conserve them (Cairns 2021).

While archeological evidence and genomic data estimate the divergence time between wolves and dogs to be ~15,000-36,000 years BP (Germonpré et al. 2009; Germonpré et

al. 2012; Larson et al. 2012; Skoglund et al. 2015; Frantz et al. 2016; Freedman and Wayne 2017), it has been estimated dingoes diverged from early dogs ~5,000-11,000 years BP (Savolainen et al. 2004; Oskarsson et al. 2012; Sacks et al. 2013; Cairns and Wilton 2016; Bergström et al. 2020; Surbakti et al. 2020; Cairns 2021; Field et al. 2022), forming a distinct and separate lineage from domestic dogs, wolves and other canids (Crowther et al. 2014; Smith et al. 2019). Research using mtDNA and Y-chromosome data has identified a close relationship between dingoes, New Guinea singing dogs (NGSD) and Asian village dogs, suggesting that the ancestors of dingoes originated in East Asia (Savolainen et al. 2004; Ardalan et al. 2012; Oskarsson et al. 2012; Sacks et al. 2013; Cairns and Wilton 2016; Cairns et al. 2017). Archeological evidence suggests dingoes arrived in Australia more than ~3,500 years BP (Milham and Thompson 1976; Savolainen et al. 2004), remaining isolated from other canids until 1788.

Although dingoes are present across different bioregions in Australia, at least two distinct mainland dingo lineages have been identified, one in the northwest and one in the southeast, alongside one offshore lineage on K'gari (formerly known as Fraser Island) (Cairns and Wilton 2016; Cairns et al. 2017; Cairns et al. 2018; Greig et al. 2018; Zhang et al. 2020). Genome-wide SNP data suggest there may be additional biogeographic structure in the mainland dingo population (Cairns et al. 2023). As the apex terrestrial predator since the extinction of the thylacine, dingoes play an important role in maintaining the ecosystems of Australia (Glen et al. 2007; Johnson et al. 2007; Letnic et al. 2009; Wallach et al. 2010; Letnic et al. 2011; Letnic et al. 2012). But they also have a complex history in Australia, as they are simultaneously declared native species and an agricultural pest dependent on jurisdiction and context. The dingo is protected in parts of

the Australian Capital Territory, Victoria, Queensland and the Northern Territory as a native species, yet at the same time, wild dogs, including dingoes, have been declared a pest or restricted matter in all Australian states and territories for posing a risk to livestock (Brink et al. 2019).

Hybridization with domestic dogs was previously highlighted as a main concern for dingo conservation, since high levels of hybridization were observed in dingoes from southeastern Australia (Newsome and Corbett 1985; Jones 1990; Corbett 2001; Wilton 2001; Elledge et al. 2008; Stephens et al. 2015; Cairns et al. 2020; Cairns 2021; Cairns et al. 2021). However, a recently published study using genome-wide SNP data has shown these occurrences are less common than previously thought (Cairns et al. 2023). Policy and legislation in Australia currently employs the term “wild dog” to refer to dingoes, feral domestic dogs and dingo-dog hybrids, assuming that pure dingoes are virtually extinct in the wild and that dingo-dog hybrids are common (Fleming 2001; DEPI 2013; Bird and Bowman 2016; NWDAP 2020). Disagreement on the dingo’s genetic identity, along with the contradictory legal status of this canid throughout Australia, have allowed for lethal control to be used on dingoes (without distinction) during pest management programmes on public and private lands across mainland Australia (van Eeden et al. 2019; Conroy et al. 2021; van Eeden et al. 2021).

K'gari is located off the coast of Queensland, Australia, and has been listed as a UNESCO World Heritage site since 1992. In addition to being the world's largest sand island, it is also home to a small protected (*Nature Conservation Act* 1992) population of dingoes of ~70-170 individuals that are genetically distinct from mainland dingoes (Brown et al. 2011; Conroy et al. 2021). The island’s Traditional owners (The Butchulla) have a deep and

long standing connection with dingoes (wongari) and in contemporary times are an infamous tourist attraction for the over 400,000 tourists visit K'gari each year (Wardell-Johnson et al. 2015), which has led to negative human-dingo interactions arising from human associated activities (O'Neill et al. 2017; Behrendorff 2021). In response to these incidents, management strategies have been implemented in K'gari, including non-lethal options as well as the removal of dingoes via lethal control (Behrendorff 2021). K'gari dingoes are of particular interest as there are concerns regarding their persistence due to low genetic diversity and inbreeding given it is a small, isolated population (Cairns et al. 2018; Conroy et al. 2021).

Isolated populations often experience reduced fitness resulting from increased levels of homozygosity and a high load of deleterious mutations across their genomes. There have been several instances in which these factors have led to inbreeding depression and a high risk of extinction. Such is the case of the iconic Isle Royale wolves, which are highly inbred gray wolves located on an island in Lake Superior, in northern United States (Hedrick et al. 2019; Robinson et al. 2019). Other examples of isolated populations in the wild that have experienced inbreeding depression are Florida Panthers (Roelke et al. 1993) and Scandinavian gray wolves (Liberg et al. 2005; Räikkönen et al. 2006; Kardos et al. 2018). In other instances, small isolated populations have managed to persist with low genetic diversity for generations, without experiencing inbreeding depression. Such is the case of island foxes (Robinson et al. 2016; Robinson et al. 2018), Appenine brown bears (Benazzo et al. 2017), African mountain gorillas (Xue et al. 2015), and Alpine ibex (Grossen et al. 2020). It has been suggested that the purging of strongly deleterious

121 recessive mutations may facilitate the persistence of long-term small populations (Xue et
122 al. 2015; Robinson et al. 2018; Grossen et al. 2020; Wootton et al. 2023).

123 Only a handful of studies have looked at the genetics of K'gari dingoes. While these
124 studies have found low genetic diversity and high levels of inbreeding on K'gari dingoes
125 (Cairns and Wilton 2016; Cairns et al. 2018; Conroy et al. 2021), more research is needed
126 to understand the consequences of inbreeding and isolation on this population. Moreover,
127 there is a need for new and well-informed conservation strategies for dingoes in Australia,
128 given that current strategies do not consider these genetic factors. To our knowledge,
129 there is only one whole-genome sequence from a K'gari dingo individual that is publicly
130 available (Zhang et al. 2020), although the provenance of the animal is uncertain. In this
131 work we include five newly sequenced samples, and we look further into the genetics of
132 K'gari dingoes to understand the consequences of their demographic history on the
133 distribution of deleterious variations, and the implications of small population sizes and
134 isolation, to help inform conservation efforts for their long-term persistence.

135 METHODS

136 Sampling and sequencing

137 In this study we include whole-genome sequencing data from a total of 41 canids,
138 including 18 dingo samples from both K'gari (6) and mainland Australia (4 from the
139 Southeast, and 8 from the Northeast). We also include 2 New Guinea singing dog
140 samples, 11 gray wolf samples, 1 red wolf sample, and 9 village dog samples.
141 Sequencing data from 3 dingo samples from mainland Australia (2 from the Northwest,
142 and 1 from the Southeast) were obtained from a recently published study (Kumar et al.

143 2023). Ten dingo samples, comprising 1 from K'gari and 9 from mainland Australia (6
144 from the Northwest, and 3 from the Southeast), were obtained from a previous study
145 (Zhang et al. 2020). Eleven gray wolf samples, 1 red wolf sample, 2 New Guinea singing
146 dog samples, and 9 village dog samples were obtained from a different study (Plassais
147 et al. 2019). Reads from all of these canids were aligned to the CanFam3 reference
148 genome using BWA-MEM (Li and Durbin 2009). Five of the K'gari dingo samples in this
149 study are from new sequencing data. Biological samples from these individuals were
150 collected between 2011 and 2013 from regions along the east coast of K'gari, including
151 Cathedral Beach, Eurong, and Dundubara. Two of the three newly sequenced dingo
152 samples from the mainland were collected from the Northwest, and one from the
153 Southeast. These samples were sequenced using Illumina Hiseq 2000, and aligned to
154 the CanFam3 reference genome using BWA-MEM (Li and Durbin 2009). We converted
155 the mapped reads in the sequencing alignment mapped (SAM) format to binary
156 alignment/mapped (BAM) format using samtools v1.3.1 (Danecek et al. 2021). Later, we
157 sorted the BAM file based on chromosomes and alignment position. These sorted reads
158 were used for marking PCR duplicates using the Picard tool (Broad Institute 2019).

159 **Genotype calling, filtering, and phasing**

160 All samples were jointly genotyped with GATK v4.2 modules HaplotypeCaller,
161 CombineGVCFs and GenotypeGVCFs. The VCF file containing raw genotype calls for all
162 41 canids were then filtered in the following way. Only sites with biallelic snps and only
163 sites with a site-level quality score QUAL > 50 were retained. Any individual genotype
164 with GQ <= 19 was set to missing. All sites with >= 10% missing data were removed. All

sites with no variation among the 41 samples (i.e., monomorphic sites) were removed. This resulted in 3,745,051 sites.

Samples were then split into three separate VCF files: all wolves (n=12), all village dogs (n=9), and all dingoes plus NGSD (n=20). Each group-level VCF was filtered separately, in the following way. As a result of small sample sizes, formal statistical tests for excess heterozygosity have no power. Therefore, to filter for excess heterozygosity, only sites where heterozygote calls made up < 75% within each species-level VCF were retained. This resulted in 3,729,028 sites for wolves, 3,649,859 sites for village dogs, and 3,733,743 sites for dingoes plus NGSD. Finally, all samples were merged back together by intersecting all sites among the three data sets, resulting in 3,632,373 sites.

SHAPEIT2 (Delaneau et al. 2014) was used to phase the genotypes of all 41 canids, using the pedigree-based recombination map from Campbell, et al. (Campbell et al. 2016). The ancestral and derived states of each variant for the 18 dingo samples was taken from Kumar et al. (Kumar et al. 2023).

Relatedness and Population Structure

We used BCFtools (Danecek et al. 2021) to remove sites with 2 or fewer minor allele counts in the 18 dingo samples, which resulted in 887,759 biallelic loci. We then used ASD (Szpiech 2014) to compute the proportion of sites at which a pair of individuals shared 0, 1, and 2 alleles Identical-By-State (IBS) for each pair of dingoes.

To assess relatedness among K'gari dingoes we plotted IBS0 (the proportion of 0 identical alleles at a given position being shared by two individuals) vs IBS2 (the proportion of 2 identical alleles being shared by two individuals). Based on records from

187 Queensland Parks and Wildlife Service (QPWS), there are three samples in the K'gari
188 dingo population that look to be related to one another by first-degree relationship (i.e.,
189 samples Y2, B44, and Y4). To verify these relationships, we used the KING method
190 (Manichaikul et al. 2010), which incorporates an algorithm that estimates a kinship
191 coefficient between pairs of individuals independent of the population's allele frequencies.

192 To assess population structure among canids we computed pairwise allele sharing
193 dissimilarities (Szpiech 2014) for all 41 canids included in this study and decomposed the
194 dissimilarity matrix with multidimensional scaling (MDS). For MDS analysis using only the
195 18 dingo samples, we subsetted the allele sharing dissimilarities from the 41 canids and
196 recomputed the MDS.

197 **Genetic diversity within and between canid populations**

198 We estimated allelic richness, private allelic richness, and the number of alleles private
199 to pairs of populations (found in each population but no other) using ADZE (Szpiech et
200 al. 2008). ADZE uses a rarefaction approach to compare these statistics at standardized
201 sample sizes. For these analyses we grouped the two mainland dingo populations
202 (northwest and southeast) and excluded the red wolf and NGSD samples because of
203 small sample sizes. Parameters were set as follows: COMB 1, K_RANGE 2, and MAX_G
204 24. To visualize results, we used R (R Core Team 2022) to plot the mean of each estimate
205 calculated for each standardized sample size.

206 **Estimating IBD and ROH**

207 To call IBD tracts, we used RefinedIBD (Browning and Browning 2013) to identify
208 Identical-By-Descent (IBD) segments with default parameters and the CanFam3.1

genomic map (Campbell et al. 2016). We called runs of homozygosity (ROH) in each canid population using GARLIC (Szpiech et al. 2017). Parameters were set as follows: --winsize 100, --auto-winsize, --auto-overlap-frac, --size-bounds 1000000 2000000 3000000 4000000 5000000, --gl-type GQ, --resample 40. This results in 6 length classes of ROH: <1Mbp, ≥1Mbp to <2Mbp, ≥2Mbp to <3Mbp, ≥3Mbp to <4Mbp, ≥4Mbp to <5Mbp, and ≥5Mbp.

As GARLIC uses allele frequency information to call ROH, we account for the close relatedness among the K'gari dingo samples, which could result in miscalling ROH in these individuals, by using the best linear unbiased estimator for allele frequencies (McPeek et al. 2004) computed from pooling all dingoes together. The allele frequency at locus i, f_i, is computed as $f_i = \sum_{k=1}^{n} \frac{\sum_{j=1}^{n} (K^{-1})_{jk}}{1^T K^{-1} 1} P_k^{(i)}$, where $P_k^{(i)}$ is the fraction of derived alleles at a locus in individual k, K is a symmetric matrix of kinship coefficients, K^{-1} is the inverse matrix of K, 1 is a column vector of n elements with all entries equal to 1, and 1^T is the transpose of 1. The kinship matrix for dingoes obtained from the --make-king option in PLINK (Purcell et al. 2007), and any negative entries were set to 0 (https://github.com/szpiech/blue_af). The allele frequencies were passed to GARLIC with the --freq-file option for use in calling ROH for the 18 dingoes and the 2 New Guinea singing dog samples.

Results were filtered to keep only the ROH segments longer than 0.5Mbp. We summarized the number of ROH segments (nROH) and sum total length of ROH (sROH) within each individual and each length class. The fraction of the genome within ROH was calculated as the total length of ROH of 0.5 Mbp or longer within an individual and divided

231 by the total length of the CanFam3.1 autosomal reference genome (2,203,764,842
232 nucleotides). Similarly, we estimated inbreeding coefficients as given by ROH for each
233 population (F_{ROH}). This coefficient represents the proportion of the autosomal genome
234 covered by ROH 1Mbp or longer.

235 **Assessing burden of deleterious variants**

236 Variants called across all 41 canids were annotated with Ensembl Variant Effect Predictor
237 (VEP) (McLaren et al. 2010). We used SIFT (Vaser et al. 2016) to predict whether an
238 alternate allele at a coding mutation is tolerated or deleterious for protein function based
239 on the CanFam3.1 genome annotations. We sort these mutations into three categories:
240 Deleterious, Tolerated, and Loss-of-Function (LoF). The “Deleterious” group includes
241 only nonsynonymous variants that were predicted as damaging (1,921 sites). The
242 “Tolerated” group contains all synonymous and nonsynonymous variants predicted as
243 tolerated (16,380 sites). If a mutation has more than one prediction we retain the most
244 damaging prediction, i.e., if there is at least one damaging prediction among multiple
245 predictions, it is classified as “Deleterious”. LoF mutations are classified as any mutation
246 with a “Stop-Gain”, “Stop-Loss”, or “Start-Loss” classification (146 sites).

247 We generated a second set of SIFT predictions, this time, requiring that the derived allele
248 is the one with the prediction. For comparison purposes, we kept both mainland dingo
249 populations (northwest and southeast) together. We created the same three categories
250 (1,357 Deleterious, 7,713 Tolerated, and 88 LoF sites) and calculated the proportion of
251 derived allele counts for each category on each dingo population.

252 To assess purging of deleterious variants we compute the Rxy statistic (Xue et al. 2015),
253 which compares the derived allele frequencies of one set of variant sites (e.g., putatively
254 deleterious sites) normalized by a set of putatively neutral sites (e.g., intergenic sites)
255 between two populations X and Y. We set X = K'gari dingoes and Y = Mainland dingoes,
256 and considered several sets of sites, including SIFT-classified Deleterious, SIFT-
257 classified Tolerated, LoF, and sites annotated as synonymous (n = 4708),
258 nonsynonymous (n = 4668), or intergenic sites (n = 10,000). For each of these sets of
259 sites, we normalize against a second (independent) set of intergenic sites (n= 10,000).
260 To account for the close relatedness among K'gari dingoes we implement a kinship-
261 weighted Rxy statistic. The modified Rxy statistic (https://github.com/szpiech/Rxy-kin)
262 incorporates the kinship matrix for K'gari and mainland dingoes obtained from the --make-
263 king option in PLINK (Purcell et al. 2007), where any negative entries are set to 0. The
264 statistic is defined (Xue et al. 2015) as

265 $R_{X,Y}(D) = \frac{L_{X,Y}(D)}{L_{Y,X}(D)}$, where $L_{A,B}(D) = \frac{\sum_{i \in D} f_i^A(1-f_i^B)}{\sum_{j \in N} f_j^A(1-f_j^B)}$, D is a set of test sites, N is a set of
266 putatively neutral sites, f_m^Z is the derived allele frequency at site m in population Z. To
267 account for relatedness when estimating allele frequencies, f_i are computed as described
268 above, using weights derived from the kinship matrix. We estimate the variance in our
269 kinship-weighted Rxy statistic using a jackknife sampling of 2,000 iterations with 20%
270 reduction per iteration.

271 # RESULTS

272 **Canid Sampling, Relatedness and Population Structure**

273 Previous analysis on dingoes suggest there are at least three distinct lineages of dingoes
274 in Australia, with one being located on the northwest of the mainland, another in the
275 southeast of the mainland, and the third one offshore on K'gari (Fraser Island) (Cairns
276 and Wilton 2016; Cairns et al. 2017; Cairns et al. 2018). To expand our understanding of
277 genetic diversity patterns in the K'gari population, we generated high quality whole-
278 genome sequences of five dingoes with average read depth between 12.01x and 12.56x
279 sampled from the island between 2011 and 2013. Although there is a dingo reference
280 genome (ASM325472v1), the newly sequenced samples in this study were aligned to the
281 dog reference genome (CanFam3) to take advantage of its fully assembled chromosomes
282 and better gene annotations. We include whole genome sequences from dingoes from
283 northwest and southeast mainland Australia, and we also include samples from other
284 canids, such as wolves and village dogs, and New Guinea singing dogs (NGSD), which
285 are a close relative of dingoes. All canid reads in this study were aligned to the dog
286 reference genome (CanFam3).

287 We identified close relatives in K'gari dingoes in two ways. First, we looked at the
288 proportion of sites at which pairs of individuals share 0, 1 or 2 alleles that are considered
289 identical-by-state (IBS). We expect Parent/Offspring to share at least one allele at a locus
290 identical-by-descent, thus having low values of IBS0. We expect full-siblings to share 2
291 alleles identical-by-descent for approximately 25% of loci, and we expect second-degree
292 relatives and unrelated individuals to have higher estimates of IBS0. We also used the
293 KING algorithm (Manichaikul et al. 2010) to infer kinship coefficients for all dingo
294 individuals. We identified three pairs of Parent/Offspring relatives, four pairs of full-siblings
295 relatives, a pair of avuncular relatives, and seven pairs that include third-degree relatives

296 and unrelated individuals. Furthermore, we found that none of the K'gari dingo individuals
297 are closely related to dingo individuals from the mainland, and that the mainland dingoes
298 in this study are not closely related to one another (Suppl. Fig. 1 and Suppl. Table 1).

299 We computed pairwise allele sharing dissimilarities to assess population structure and
300 generated multidimensional scaling (MDS) plots from the dissimilarity matrix. In MDS,
301 individuals with small allele sharing dissimilarities tend to group together. In fig. 1A we
302 can observe distinct taxonomic groupings, with the close relationship between the dingo
303 and NGSD lineages clearly captured in this analysis. Individuals within the wolf and village
304 dog populations were sampled from different regions around the world (see Suppl. Table
305 2), hence the relatively loose clustering among these individuals. Interestingly, we find
306 that the village dog from Vietnam seems to be genetically more similar to mainland
307 dingoes than the village dog from Papua New Guinea (fig. 1A). While Papua New Guinea
308 is geographically closer to Australia than Vietnam, similar observations regarding shared
309 ancestry between Vietnamese village dogs and dingoes have been reported by Cairns et
310 al. (Cairns et al. 2023). A possible explanation regarding the observed genotype
311 differences among these canids may be explained by Vietnamese village dogs having
312 retained the ancestral alleles that also exist in the dingo lineage while village dogs from
313 Papua New Guinea have not, possibly because of their different demographic histories
314 or genetic swamping from contemporary domestic dogs.

315 Although dingoes from the southeast and northwest overlap with each other (i.e., they are
316 genetically more similar), we observe K'gari dingoes form their own separate subgroup
317 (fig 1A). To look more into detail at the allele sharing differences between the three dingo
318 populations, we recomputed the MDS on the matrix of allele sharing dissimilarities

containing only these samples. In fig. 1B we can observe geographic based clustering, with K'gari dingoes maintaining a tighter and more distinct group compared to dingoes in the southeast and northwest.

Genetic diversity between and within canid populations

To characterize genetic diversity patterns within and between canid populations we analyzed the distribution of alleles across populations. We used ADZE (Szpiech et al. 2008) to compute estimates of allelic richness, private allelic richness, and the number of alleles shared by pairs of populations (i.e., alleles that are found only in those populations and nowhere else). We observed reduced levels of genetic diversity in mainland and K'gari dingoes, compared to large and outbred populations such as wolves and village dogs, with K'gari dingoes having the lowest genetic diversity of all the canid groups analyzed in this study (fig. 2A and 2B). We found mainland dingoes share more alleles with village dogs than K'gari dingoes and village dogs (fig. 2C), which suggests that the alleles shared between dingoes and their common ancestors from Southeast Asia were likely lost in the bottleneck that founded the K'gari dingo population.

Inbreeding and homozygosity

To characterize the levels of inbreeding we looked at long contiguous stretches of homozygous regions in individual genomes that are shared identical-by-descent (IBD), also known as runs of homozygosity (ROH). We used GARLIC (Szpiech et al. 2017) to call ROH, which implements a likelihood-based approach to infer ROH. The length of ROH can inform on how recent the parental relatedness among individuals is, with long ROH more commonly arising in offspring descended from parents with a recent common

341 ancestor. On the other hand, short ROH suggests a more distant common ancestor, as
342 recombination has had more time to break apart the underlying IBD haplotypes (Kirin et
343 al. 2010; Pemberton et al. 2012). The wolf and village dog individuals included in this
344 study were sampled from large and outbred populations from different regions around the
345 world (see suppl. Table 2 for details), resulting in variation in the levels of homozygosity
346 found in their individual genomes. Overall, we observed relatively low levels of
347 homozygosity in village dogs and wolves, with ~7% and ~16% of their genomes being
348 covered by ROH >0.5 Mbp, respectively (fig. 3). In contrast, we observed high levels of
349 homozygosity in Northwest and Southeast mainland dingo populations, with ~30% of their
350 genomes being covered by ROH >0.5 Mbp. In K'gari dingoes we observed even higher
351 levels of homozygosity, with ~65% of their genomes being covered by ROH >0.5 Mbp,
352 and ~28% by ROH >5 Mbp (fig. 3), with the longest individual homozygous segment being
353 64.2 Mbp long (suppl. Fig 3).

354 We estimated inbreeding coefficients as given by ROH for each population (F_{ROH}), which
355 represents the proportion of the autosomal genome covered by ROH >1Mbp. F_{ROH} of
356 0.59, 0.23, 0.23, were estimated for K'gari, Northwest, and Southeast dingoes,
357 respectively, and F_{ROH} of 0.35 for NGSD. For village dogs and wolves we estimated
358 inbreeding coefficients of 0.04 and 0.13, respectively. These results provide clear
359 evidence for inbreeding, isolation, small population size, and a strong founder effect in
360 K'gari dingoes.

361 **Patterns of deleterious variation and genetic load**

362 To understand the effect of inbreeding and low genetic diversity on the distribution of
363 deleterious variation, we looked at the burden of deleterious alleles in K'gari dingoes
364 compared to mainland dingoes and other canids. Alternate alleles were predicted to be
365 deleterious or neutral by taking into account sequence conservation and the effect of an
366 amino acid substitution on protein function using SIFT (Vaser et al. 2016) (see Methods).
367 We found dingoes and NGSD carry a higher burden of deleterious alternate-allele
368 homozygotes compared to village dogs and wolves, although K'gari dingoes carry the
369 highest proportion of deleterious alternate-allele homozygotes among all the canids
370 included in this study (fig. 4A). On the other hand, both mainland and K'gari dingoes seem
371 to carry similar proportions of alternate-allele homozygous LoF variants (fig. 4B).

372 To refine our analysis of the differences in the burden of deleterious alleles between K'gari
373 and mainland dingoes, we subset our deleterious alternate allele calls to include only
374 those alternate alleles which were also inferred to be the derived allele in dingoes (see
375 Methods). We computed proportions for deleterious and LoF derived variants in each
376 dingo sample. Patterns of deleterious/LoF derived homozygotes and heterozygotes
377 stayed qualitatively similar to the non-polarized patterns. We computed the proportion of
378 total deleterious/LoF derived alleles per individual by counting the number of
379 deleterious/LoF heterozygotes and two times the number of deleterious/LoF
380 homozygotes (divided by two times the total number of genotypes). Both K'gari and
381 mainland dingoes showed a similar proportion of predicted deleterious alleles (sample
382 means 290 and 278, respectively, p-value > 0.05 by Mann-Whitney U test; suppl. Fig 4A),
383 which suggests these alleles may not be strongly deleterious as they have not been
384 removed from the dingo population in K'gari through negative selection. In contrast, we

385 observed a significant difference in the proportion of derived allele counts for LoF variants
386 (sample means 14 and 19 for K'gari and mainland dingoes, respectively, p-value =
387 0.001101 by Mann-Whitney U test), with a decrease in K'gari dingoes compared to
388 mainland dingoes (suppl. Fig 4B). Moreover, the mean number of homozygous LoF
389 variants per individual in K'gari dingoes is significantly lower than in mainland dingoes
390 (6.17 and 5.25, respectively, p-value = 0.017 by Mann-Whitney U test). Similar patterns
391 have been observed in other small populations with substantial inbreeding, suggesting
392 purging of LoF alleles, which are more likely to be strongly recessive variants than small-
393 effect deleterious variants, may facilitate the persistence of small and isolated populations
394 (Robinson et al. 2018; Grossen et al. 2020).

395 To test whether K'gari dingoes showed evidence of purging of deleterious variants we
396 used the Rxy statistic, as implemented by Xue et al. (Xue et al. 2015), which compares
397 the frequency of derived alleles of a particular category (e.g., deleterious) normalized by
398 the frequencies of a set of putatively neutral variants, which is then compared across two
399 populations, X and Y. When Rxy < 1 for a given site category, we consider this evidence
400 of purging of those alleles in population X relative to Y, when Rxy > 1 we consider this
401 evidence of enrichment of those alleles in population X relative to Y. When Rxy = 1, we
402 consider this evidence of no difference of those alleles between X and Y. As our K'gari
403 samples are closely related, we implemented a modified version of the Rxy statistic, which
404 accounts for relatedness among K'gari dingoes. For this analysis we looked at several
405 allele categories: sites that were predicted as deleterious, tolerated, LoF, sites that were
406 annotated as synonymous, non-synonymous, and a random set of intergenic sites as a
407 control. All Rxy calculations used an additional, independent, set of intergenic sites for

408 normalization. We estimated the excess number of deleterious variants in K'gari dingoes
409 (population X) with respect to mainland dingoes (population Y) and found evidence of
410 purging of LoF alleles (i.e., putative strongly deleterious alleles), but little to no evidence
411 of purging of mild/slightly deleterious alleles in K'gari dingoes (fig. 5). Although we
412 observe a small enrichment in the rest of the categories, we hypothesize these results
413 may be due to the small sample size and low genetic diversity in K'gari dingoes compared
414 to mainland dingoes. To assess the effect of sample sizes on our results, we conducted
415 this analysis using the same number of samples for each dingo population and found that
416 results stayed very similar (Suppl. Fig. 5).

417 DISCUSSION

418 In this work, we have generated five whole-genome sequences of K'gari dingoes to
419 investigate the consequences of isolation and inbreeding on this island population. We
420 find K'gari dingoes have lower genetic diversity, a higher level of homozygosity, and a
421 higher accumulation of mildly deleterious alleles compared to dingoes in the mainland
422 and canids from large populations. However, contrary to the idea that inbred populations
423 are bound to experience inbreeding depression and subsequently face a high risk of
424 extinction, some populations are able to persist in small numbers and with low genetic
425 diversity (Xue et al. 2015; Benazzo et al. 2017; Robinson et al. 2018; Grossen et al. 2020).
426 Understanding the complex relationship between genetic diversity, inbreeding, mutational
427 load, and demographic history are key to leveraging this information for management and
428 conservation strategies of populations in the wild.

429 In large and outbred populations strongly deleterious recessive alleles are “hidden” from
430 selection in heterozygous form, making large populations more prone to experiencing
431 inbreeding depression after going through a harsh bottleneck (Robinson et al. 2018;
432 Kyriazis et al. 2021). On the other hand, small and isolated populations tend to have a
433 higher burden of deleterious variants as a result of inbreeding (Robinson et al. 2018;
434 Hedrick et al. 2019; Grossen et al. 2020; Khan et al. 2021; Stoffel et al. 2021). We observe
435 a similar trend in K’gari dingoes, finding higher levels of homozygosity in this population
436 compared to canids from large and stable populations, such as wolves and village dogs.

437 Accumulation of long ROH in the genome is associated with increased presence of
438 deleterious homozygotes and potential fitness effects (Szpiech et al. 2013; Szpiech et al.
439 2019; Stoffel et al. 2021). Although the levels of ROH and estimated inbreeding found in
440 K’gari dingoes (F_{ROH} = 0.59 on average; fig. 3) in some cases surpass those observed in
441 wild populations of other species already experiencing inbreeding depression (Khan et al.
442 2021; Stoffel et al. 2021; Colpitts et al. 2022), surveillance and necropsy records from the
443 Queensland Parks and Wildlife Service (QPWS) and the latest report on the genetic
444 health of K’gari dingoes (Allen 2023) have not recorded abnormal phenotypes in K’gari
445 dingoes. Nonetheless, whether a small population will experience inbreeding depression
446 or not depends on various factors, including the size of the ancestral population and the
447 magnitude and history of bottleneck events.

448 It has been observed that long-term small populations (i.e., populations that experienced
449 early bottlenecks and have maintained small population size following the bottlenecks)
450 can persist despite showing high levels of inbreeding by purging strongly recessive
451 deleterious variants (Xue et al. 2015; Benazzo et al. 2017; Robinson et al. 2018; Grossen

452 et al. 2020), whereas populations that have gone through multiple recent bottlenecks will
453 most likely experience inbreeding depression despite purging (Khan et al. 2021; Stoffel
454 et al. 2021). Previous studies have documented dingoes in K'gari have experienced at
455 least two bottlenecks in the form of large-scale culling events during the past 120 years
456 (Petrie 1995; Allen et al. 2015). Our assessment of purging in this population indicates
457 that while strongly deleterious alleles are likely being purged, there is little to no purging
458 of mildly deleterious alleles in this population. This may have important long term
459 consequences for the health of the K'gari dingoes that should be considered by
460 conservation and management programs.

461 Understanding the genetic factors that allow inbred populations to persist can aid in the
462 design and implementation of appropriate conservation and management strategies. One
463 of the main conservation strategies employed to assist highly inbred populations is
464 genetic rescue, which relies on the introduction of individuals from a large population
465 carrying genetic diversity into the inbred population. Although genetic rescue has been
466 used widely as a conservation strategy, there have been instances in which the benefits
467 have been short-lived, such is the case of Isle Royale wolves (Hedrick et al. 2014; Hedrick
468 et al. 2019). It should be noted that Isle Royale wolves are a relatively recent population,
469 having experienced fluctuations in population size ranging between ~15 and 30
470 individuals since establishing in Isle Royale around the 1940s (Peterson et al. 2014). The
471 introduction of individuals from a large population (with high genetic diversity) into this
472 inbred wolf population resulted in a higher burden of strongly deleterious variants, which
473 then led to inbreeding depression (Robinson et al. 2019; Kyriazis et al. 2021). On the
474 other hand, dingoes have inhabited Australia for at least 3,500 years (Milham and

Thompson 1976; Savolainen et al. 2004). Although the time of arrival of dingoes on K'gari remains unknown, we find dingoes from the mainland and K'gari are genetically distinct. Moreover, our results indicate that both dingo populations have lower genetic diversity compared to large and outbred populations, which could make mainland dingoes a potential source population to introduce genetic diversity in K'gari dingoes. However, closely monitoring for signs of inbreeding depression and more research (e.g. population genetic simulations) have been recommended under similar scenarios (Robinson et al. 2018; Kyriazis et al. 2021; Robinson et al. 2022) to explore the effectiveness of this strategy, given that non-genetic factors (e.g. environmental stressors) may also play a role in population persistence.

Current conservation and management programs treat dingoes as a single homogeneous population, although studies using mtDNA (Savolainen et al. 2004; Oskarsson et al. 2012; Cairns and Wilton 2016; Greig et al. 2018) and Y-chromosome (Ardalan et al. 2012; Sacks et al. 2013; Cairns et al. 2017) data as well as genome-wide SNP data (Cairns et al. 2023) and whole-genome sequencing (Zhang et al. 2020) have identified at least three distinct dingo populations. Adding to previous evidence, we find two distinct populations in the mainland (northwest and southeast), and one offshore in K'gari. However, we identify long IBD segments shared between dingoes from K'gari and Northwest Australia, and between dingoes from K'gari and the Southeast (suppl. Fig. 2). These patterns may be due to differences in female and male dingo dispersal throughout mainland Australia, with males having a broader dispersal range than females. Cairns et al. (Cairns et al. 2017) found dingoes in the Northwest and K'gari carry the paternal haplogroup from Northwest dingoes, and dingoes sampled from K'gari carry the maternal lineage from dingoes in the

Southeast. In light of this, recognizing several dingo subpopulations can help inform conservation strategies.

Although dingoes are protected on K’gari, they are euthanized due to injury, poor health, or as a result of high-risk behavior towards humans. Moreover, QPWS have documented conspecific killing (Behrendorff et al. 2018), vehicle strikes and snake bite, and young dingoes may be susceptible to predation such as goannas, snakes, and birds of prey. There is considerable debate about how the population should be managed, both in terms of conservation and how to reduce risk to tourists visiting the island. This work helps broaden our knowledge and understanding about the evolutionary history and genetic health of K'gari dingoes in order to better inform conservation and public policies. Our work, although limited by a small sample size and the sampling of several closely related samples, indicates that the dingo population on K’gari is challenged by inbreeding as a result of founder effects and successive bottlenecks. We find the first evidence that K’gari dingoes may be purging strongly deleterious mutations, whilst accumulating a higher genetic load of mild/slightly deleterious mutations. Further research is needed to investigate the genetic mutation load of dingoes on K’gari using a larger sample size, as well as biological monitoring of the population for symptoms of inbreeding depression such as reduced fertility, increased stillbirths, and congenital birth defects. QPWS and Butchulla Prescribed Body Corporates are left with the challenge of preserving dingoes on K’gari, balancing the need to address human safety concerns and protecting the dingo population's genetic health and viability into the future.

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SUPPLEMENTARY MATERIAL

Supplementary data are available at XXXX.

DATA ACCESSIBILITY

Raw sequence data of each of the K'gari dingo genomes generated in this study are available on SRA under project XXXX (TBD).

CONFLICTS OF INTEREST

KMC is a scientific advisor to the Australian Dingo Foundation, The New Guinea Singing Dog Conservation Society and The New Guinea Highland Wild Dog Foundation. KMC and LB are members of the IUCN Canid Specialist Group Dingo Working Group.

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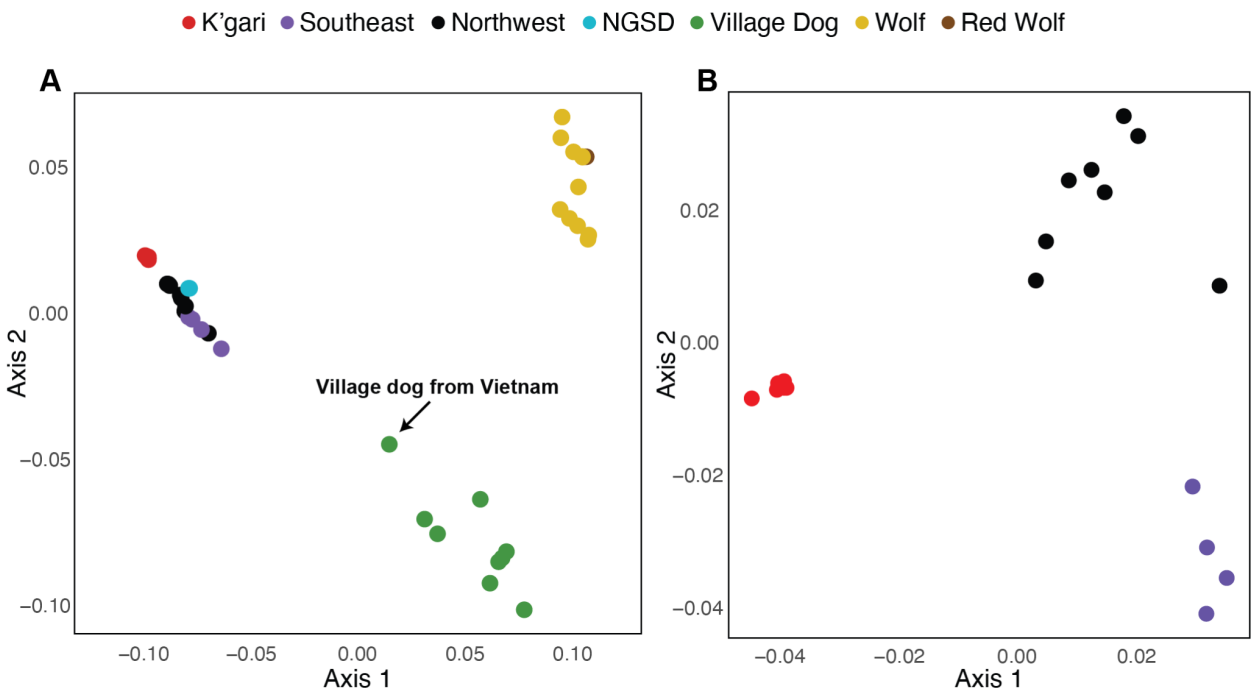
826

827 **FIGURES**



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829 Figure 1. Reduced dimensional representation of sample clustering based on

830 multidimensional scaling of the allele sharing dissimilarity matrix. First two major axes of

831 variation plotted for (A) all 41 canids included in this study and (B) 18 dingo individuals.

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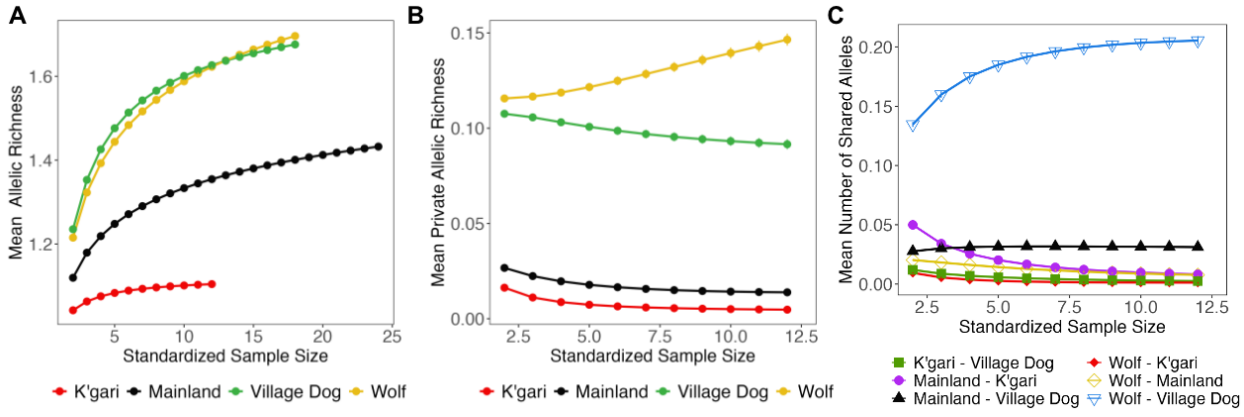

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Figure 2. Genetic diversity estimates within and between canid populations. Error bars representing 95% confidence intervals for each line are too narrow to be visible. (A) Mean number of alleles per locus (allelic richness). (B) Mean number of private alleles per locus (private allelic richness). (C) Mean number of shared alleles per locus between pairs of populations.

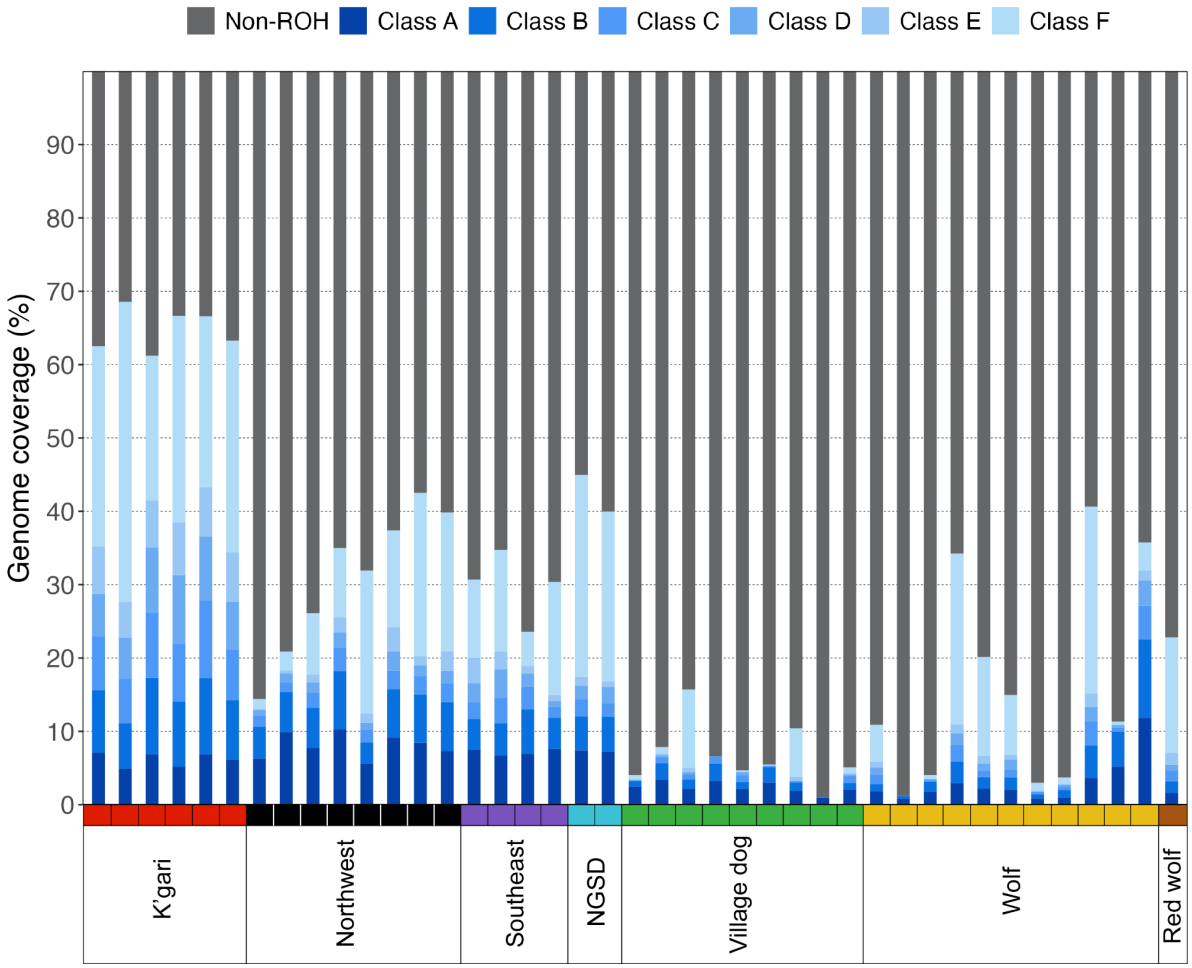

Figure 3. ROH coverage across individual genomes. The percentage of the genome covered by class A ROH (0.5-1 Mbp), class B ROH (1-2 Mbp), class C ROH (2-3 Mbp), class D ROH (3-4 Mbp), class E ROH (4-5 Mbp), and class F ROH (>5 Mbp) is shown on the Y-axis for each of the 41 canids included in this study.

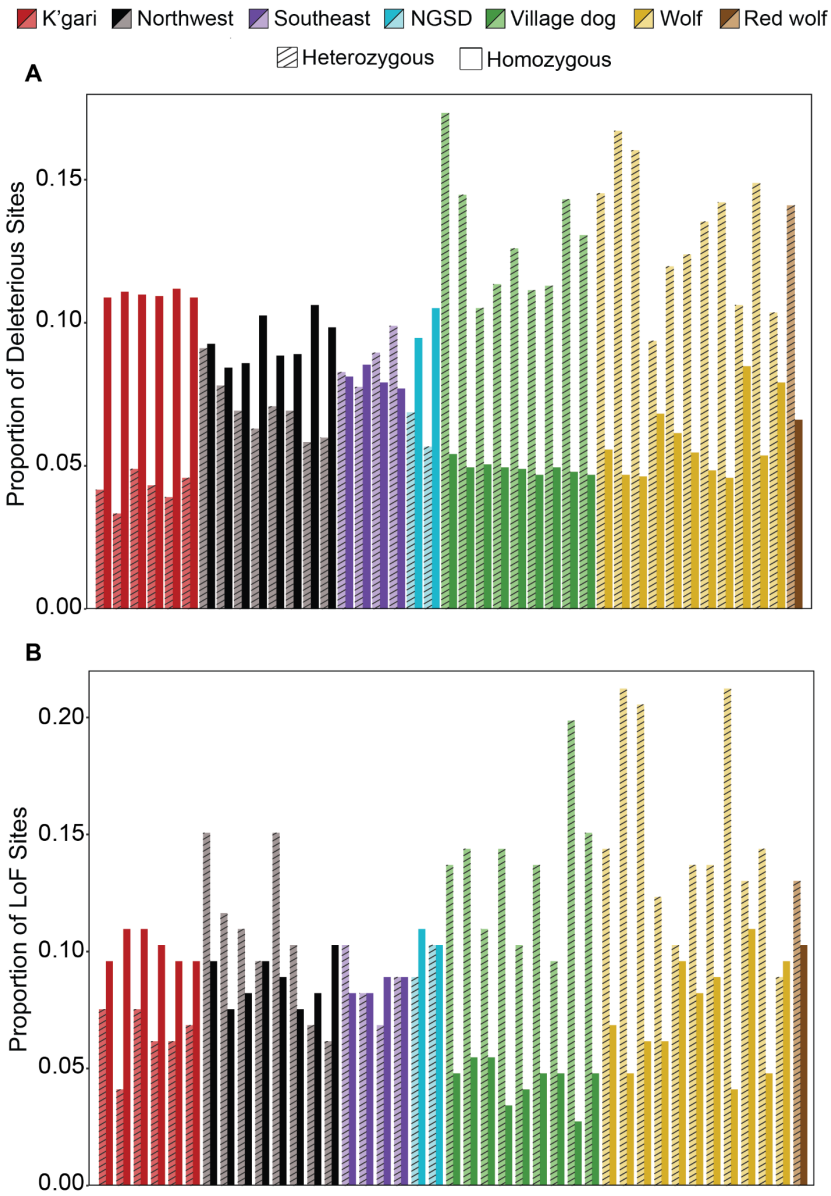

Figure 4. Predicted damaging alleles. (A) Predicted deleterious variants. (B) Loss-of-Function (LoF) variants. For each category solid bars show the proportion of homozygous sites, and striped bars show the proportion of heterozygous sites for each canid individual of each population.

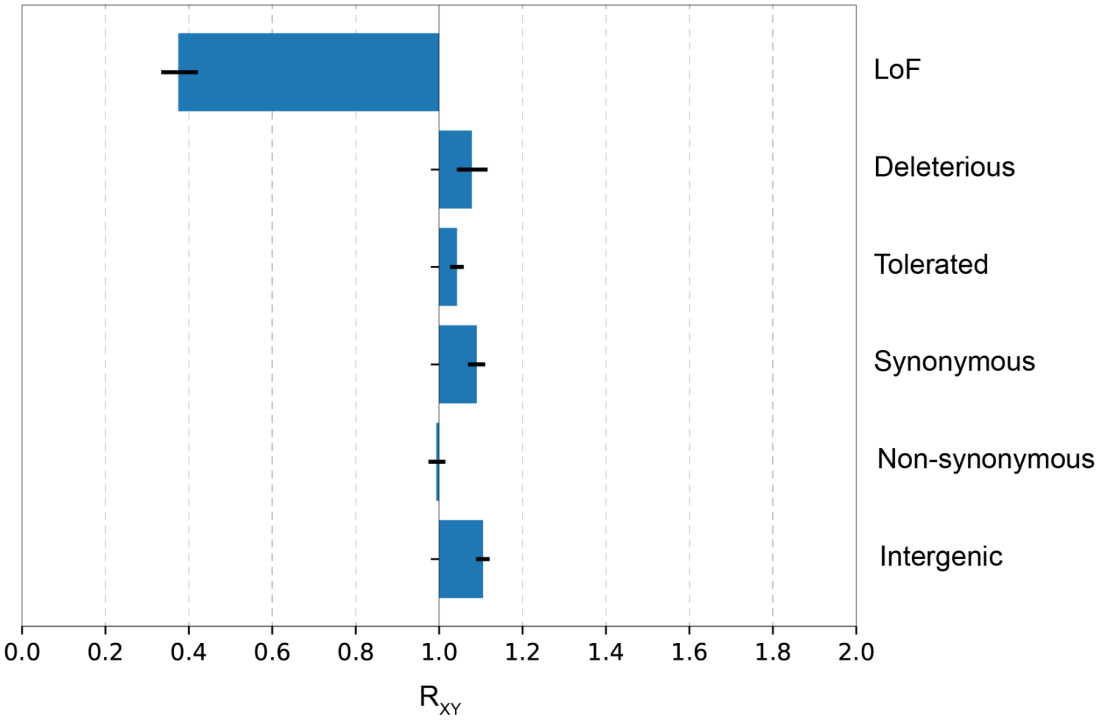

Figure 5. Kinship-weighted R_{xy} estimates for K'gari and mainland dingoes. Estimates represent the relative excess number of derived alleles for each category in K'gari dingoes (population X) relative to mainland dingoes (population Y). Error bars represent the minimum and maximum values for the 95% confidence interval from jackknife.