Genomic consequences of isolation and inbreeding in an island dingo population

Ana V. Leon-Apodaca[1], Manoharan Kumar[2], Andres del Castillo[1], Gabriel C. Conroy[3,4], Robert W. Lamont[3,4], Steven Ogbourne[4], Kylie M. Cairns[5,6], Liz Borburgh[3], Linda Behrendorff[7], Sankar Subramanian[2,4,*], Zachary A. Szpiech[1,8,*]

[1] Department of Biology, Pennsylvania State University, PA, USA

[2] School of Science, Technology & Engineering, University of the Sunshine Coast, 1 Moreton Parade, Petrie, Queensland, Australia

[3] School of Science, Technology & Engineering, University of the Sunshine Coast, 90 Sippy Downs Drive, Sippy Downs, Queensland, Australia

[4] Centre for Bioinnovation, University of the Sunshine Coast, 90 Sippy Downs Drive, Sippy Downs, Queensland, Australia

[5] Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, UNSW Australia, Sydney NSW 2052, Australia

[6] Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, UNSW Australia, Sydney NSW 2052, Australia

[7] Queensland Parks and Wildlife Service, Department of Environment & Science, K'gari, Australia

[8] Institute for Computational and Data Sciences, Pennsylvania State University, PA, USA

*Corresponding authors

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

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

METHODS

Sampling and sequencing

In this study we include whole-genome sequencing data from a total of 41 canids, including 18 dingo samples from both K'gari (6) and mainland Australia (4 from the Southeast, and 8 from the Northeast). We also include 2 New Guinea singing dog samples, 11 gray wolf samples, 1 red wolf sample, and 9 village dog samples. Sequencing data from 3 dingo samples from mainland Australia (2 from the Northwest, and 1 from the Southeast) were obtained from a recently published study (Kumar et al. 2023). Ten dingo samples, comprising 1 from K'gari and 9 from mainland Australia (6 from the Northwest, and 3 from the Southeast), were obtained from a previous study

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

158 **Genotype calling, filtering, and phasing**

159 All samples were jointly genotyped with GATK v4.2 modules HaplotypeCaller,
160 CombineGVCFs and GenotypeGVCFs. The VCF file containing raw genotype calls for all
161 41 canids were then filtered in the following way. Only sites with biallelic snps and only
162 sites with a site-level quality score QUAL > 50 were retained. Any individual genotype
163 with GQ <= 19 was set to missing. All sites with >= 10% missing data were removed. All
164 sites with no variation among the 41 samples (i.e., monomorphic sites) were removed.
165 This resulted in 3,745,051 sites.

166 Samples were then split into three separate VCF files: all wolves (n=12), all village dogs
167 (n=9), and all dingoes plus NGSD (n=20). Each group-level VCF was filtered separately,
168 in the following way. As a result of small sample sizes, formal statistical tests for excess
169 heterozygosity have no power. Therefore, to filter for excess heterozygosity, only sites
170 where heterozygote calls made up < 75% within each species-level VCF were retained.
171 This resulted in 3,729,028 sites for wolves, 3,649,859 sites for village dogs, and
172 3,733,743 sites for dingoes plus NGSD. Finally, all samples were merged back together
173 by intersecting all sites among the three data sets, resulting in 3,632,373 sites.

174 SHAPEIT2 (Delaneau et al. 2014) was used to phase the genotypes of all 41 canids,
175 using the pedigree-based recombination map from Campbell, et al. (Campbell et al.
176 2016). The ancestral and derived states of each variant for the 18 dingo samples was
177 taken from Kumar et al. (Kumar et al. 2023).

178 **Relatedness and Population Structure**

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

183 To assess relatedness among K'gari dingoes we plotted IBS0 (the proportion of 0
184 identical alleles at a given position being shared by two individuals) vs IBS2 (the
185 proportion of 2 identical alleles being shared by two individuals). Based on records from
186 Queensland Parks and Wildlife Service (QPWS), there are three samples in the K'gari
187 dingo population that look to be related to one another by first-degree relationship (i.e.,

188 samples Y2, B44, and Y4). To verify these relationships, we used the KING method
189 (Manichaikul et al. 2010), which incorporates an algorithm that estimates a kinship
190 coefficient between pairs of individuals independent of the population's allele frequencies.

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

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

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

205 **Estimating IBD and ROH**

206 To call IBD tracts, we used RefinedIBD (Browning and Browning 2013) to identify
207 Identical-By-Descent (IBD) segments with default parameters and the CanFam3.1
208 genomic map (Campbell et al. 2016). We called runs of homozygosity (ROH) in each
209 canid population using GARLIC (Szpiech et al. 2017). Parameters were set as follows: --

210 winsize 100, --auto-winsize, --auto-overlap-frac, --size-bounds 1000000 2000000
211 3000000 4000000 5000000, --gl-type GQ, --resample 40. This results in 6 length classes
212 of ROH: <1Mbp, ≥1Mbp to <2Mbp, ≥2Mbp to <3Mbp, ≥3Mbp to <4Mbp, ≥4Mbp to <5Mbp,
213 and ≥5Mbp.

214 As GARLIC uses allele frequency information to call ROH, we account for the close
215 relatedness among the K'gari dingo samples, which could result in miscalling ROH in
216 these individuals, by using the best linear unbiased estimator for allele frequencies
217 (McPeek et al. 2004) computed from pooling all dingoes together. The allele frequency at
218 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
219 alleles at a locus in individual k, K is a symmetric matrix of kinship coefficients, K^{-1} is the
220 inverse matrix of K, 1 is a column vector of n elements with all entries equal to 1, and 1^T
221 is the transpose of 1. The kinship matrix for dingoes obtained from the --make-king option
222 in PLINK (Purcell et al. 2007), and any negative entries were set to 0
223 (https://github.com/szpiech/blue_af). The allele frequencies were passed to GARLIC with
224 the --freq-file option for use in calling ROH for the 18 dingoes and the 2 New Guinea
225 singing dog samples.

226 Results were filtered to keep only the ROH segments longer than 0.5Mbp. We
227 summarized the number of ROH segments (nROH) and sum total length of ROH (sROH)
228 within each individual and each length class. The fraction of the genome within ROH was
229 calculated as the total length of ROH of 0.5 Mbp or longer within an individual and divided
230 by the total length of the CanFam3.1 autosomal reference genome (2,203,764,842
231 nucleotides). Similarly, we estimated inbreeding coefficients as given by ROH for each

232 population (F_{ROH}). This coefficient represents the proportion of the autosomal genome
233 covered by ROH 1Mbp or longer.

234 **Assessing burden of deleterious variants**

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

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

251 To assess purging of deleterious variants we compute the Rxy statistic (Xue et al. 2015),
252 which compares the derived allele frequencies of one set of variant sites (e.g., putatively
253 deleterious sites) normalized by a set of putatively neutral sites (e.g., intergenic sites)

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

264 $R_{X,Y}(D) = \frac{L_{X,Y}(D)}{L_{Y,X}(D)}$, 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
265 putatively neutral sites, f_m^Z is the derived allele frequency at site m in population Z. To
266 account for relatedness when estimating allele frequencies, f_i are computed as described
267 above, using weights derived from the kinship matrix. We estimate the variance in our
268 kinship-weighted Rxy statistic using a jackknife sampling of 2,000 iterations with 20%
269 reduction per iteration.

270 RESULTS

271 Canid Sampling, Relatedness and Population Structure

272 Previous analysis on dingoes suggest there are at least three distinct lineages of dingoes
273 in Australia, with one being located on the northwest of the mainland, another in the
274 southeast of the mainland, and the third one offshore on K'gari (Fraser Island) (Cairns

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

286 We identified close relatives in K'gari dingoes in two ways. First, we looked at the
287 proportion of sites at which pairs of individuals share 0, 1 or 2 alleles that are considered
288 identical-by-state (IBS). We expect Parent/Offspring to share at least one allele at a locus
289 identical-by-descent, thus having low values of IBS0. We expect full-siblings to share 2
290 alleles identical-by-descent for approximately 25% of loci, and we expect second-degree
291 relatives and unrelated individuals to have higher estimates of IBS0. We also used the
292 KING algorithm (Manichaikul et al. 2010) to infer kinship coefficients for all dingo
293 individuals. We identified three pairs of Parent/Offspring relatives, four pairs of full-siblings
294 relatives, a pair of avuncular relatives, and seven pairs that include third-degree relatives
295 and unrelated individuals. Furthermore, we found that none of the K'gari dingo individuals
296 are closely related to dingo individuals from the mainland, and that the mainland dingoes
297 in this study are not closely related to one another (Suppl. Fig. 1 and Suppl. Table 1).

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

314 Although dingoes from the southeast and northwest overlap with each other (i.e., they are
315 genetically more similar), we observe K'gari dingoes form their own separate subgroup
316 (fig 1A). To look more into detail at the allele sharing differences between the three dingo
317 populations, we recomputed the MDS on the matrix of allele sharing dissimilarities
318 containing only these samples. In fig. 1B we can observe geographic based clustering,
319 with K'gari dingoes maintaining a tighter and more distinct group compared to dingoes in
320 the southeast and northwest.

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

322 To characterize genetic diversity patterns within and between canid populations we
323 analyzed the distribution of alleles across populations. We used ADZE (Szpiech et al.
324 2008) to compute estimates of allelic richness, private allelic richness, and the number of
325 alleles shared by pairs of populations (i.e., alleles that are found only in those populations
326 and nowhere else). We observed reduced levels of genetic diversity in mainland and
327 K'gari dingoes, compared to large and outbred populations such as wolves and village
328 dogs, with K'gari dingoes having the lowest genetic diversity of all the canid groups
329 analyzed in this study (fig. 2A and 2B). We found mainland dingoes share more alleles
330 with village dogs than K'gari dingoes and village dogs (fig. 2C), which suggests that the
331 alleles shared between dingoes and their common ancestors from Southeast Asia were
332 likely lost in the bottleneck that founded the K'gari dingo population.

333 **Inbreeding and homozygosity**

334 To characterize the levels of inbreeding we looked at long contiguous stretches of
335 homozygous regions in individual genomes that are shared identical-by-descent (IBD),
336 also known as runs of homozygosity (ROH). We used GARLIC (Szpiech et al. 2017) to
337 call ROH, which implements a likelihood-based approach to infer ROH. The length of
338 ROH can inform on how recent the parental relatedness among individuals is, with long
339 ROH more commonly arising in offspring descended from parents with a recent common
340 ancestor. On the other hand, short ROH suggests a more distant common ancestor, as
341 recombination has had more time to break apart the underlying IBD haplotypes (Kirin et
342 al. 2010; Pemberton et al. 2012). The wolf and village dog individuals included in this

343 study were sampled from large and outbred populations from different regions around the
344 world (see suppl. Table 2 for details), resulting in variation in the levels of homozygosity
345 found in their individual genomes. Overall, we observed relatively low levels of
346 homozygosity in village dogs and wolves, with ~7% and ~16% of their genomes being
347 covered by ROH >0.5 Mbp, respectively (fig. 3). In contrast, we observed high levels of
348 homozygosity in Northwest and Southeast mainland dingo populations, with ~30% of their
349 genomes being covered by ROH >0.5 Mbp. In K'gari dingoes we observed even higher
350 levels of homozygosity, with ~65% of their genomes being covered by ROH >0.5 Mbp,
351 and ~28% by ROH >5 Mbp (fig. 3), with the longest individual homozygous segment being
352 64.2 Mbp long (suppl. Fig 3).

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

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

361 To understand the effect of inbreeding and low genetic diversity on the distribution of
362 deleterious variation, we looked at the burden of deleterious alleles in K'gari dingoes
363 compared to mainland dingoes and other canids. Alternate alleles were predicted to be
364 deleterious or neutral by taking into account sequence conservation and the effect of an

365 amino acid substitution on protein function using SIFT (Vaser et al. 2016) (see Methods).
366 We found dingoes and NGSD carry a higher burden of deleterious alternate-allele
367 homozygotes compared to village dogs and wolves, although K'gari dingoes carry the
368 highest proportion of deleterious alternate-allele homozygotes among all the canids
369 included in this study (fig. 4A). On the other hand, both mainland and K'gari dingoes seem
370 to carry similar proportions of alternate-allele homozygous LoF variants (fig. 4B).

371 To refine our analysis of the differences in the burden of deleterious alleles between K'gari
372 and mainland dingoes, we subset our deleterious alternate allele calls to include only
373 those alternate alleles which were also inferred to be the derived allele in dingoes (see
374 Methods). We computed proportions for deleterious and LoF derived variants in each
375 dingo sample. Patterns of deleterious/LoF derived homozygotes and heterozygotes
376 stayed qualitatively similar to the non-polarized patterns. We computed the proportion of
377 total deleterious/LoF derived alleles per individual by counting the number of
378 deleterious/LoF heterozygotes and two times the number of deleterious/LoF
379 homozygotes (divided by two times the total number of genotypes). Both K'gari and
380 mainland dingoes showed a similar proportion of predicted deleterious alleles (sample
381 means 290 and 278, respectively, p-value > 0.05 by Mann-Whitney U test; suppl. Fig 4A),
382 which suggests these alleles may not be strongly deleterious as they have not been
383 removed from the dingo population in K'gari through negative selection. In contrast, we
384 observed a significant difference in the proportion of derived allele counts for LoF variants
385 (sample means 14 and 19 for K'gari and mainland dingoes, respectively, p-value =
386 0.001101 by Mann-Whitney U test), with a decrease in K'gari dingoes compared to
387 mainland dingoes (suppl. Fig 4B). Moreover, the mean number of homozygous LoF

388 variants per individual in K'gari dingoes is significantly lower than in mainland dingoes
389 (6.17 and 5.25, respectively, p-value = 0.017 by Mann-Whitney U test). Similar patterns
390 have been observed in other small populations with substantial inbreeding, suggesting
391 purging of LoF alleles, which are more likely to be strongly recessive variants than small-
392 effect deleterious variants, may facilitate the persistence of small and isolated populations
393 (Robinson et al. 2018; Grossen et al. 2020).

394 To test whether K'gari dingoes showed evidence of purging of deleterious variants we
395 used the Rxy statistic, as implemented by Xue et al. (Xue et al. 2015), which compares
396 the frequency of derived alleles of a particular category (e.g., deleterious) normalized by
397 the frequencies of a set of putatively neutral variants, which is then compared across two
398 populations, X and Y. When Rxy < 1 for a given site category, we consider this evidence
399 of purging of those alleles in population X relative to Y, when Rxy > 1 we consider this
400 evidence of enrichment of those alleles in population X relative to Y. When Rxy = 1, we
401 consider this evidence of no difference of those alleles between X and Y. As our K'gari
402 samples are closely related, we implemented a modified version of the Rxy statistic, which
403 accounts for relatedness among K'gari dingoes. For this analysis we looked at several
404 allele categories: sites that were predicted as deleterious, tolerated, LoF, sites that were
405 annotated as synonymous, non-synonymous, and a random set of intergenic sites as a
406 control. All Rxy calculations used an additional, independent, set of intergenic sites for
407 normalization. We estimated the excess number of deleterious variants in K'gari dingoes
408 (population X) with respect to mainland dingoes (population Y) and found evidence of
409 purging of LoF alleles (i.e., putative strongly deleterious alleles), but little to no evidence
410 of purging of mild/slightly deleterious alleles in K'gari dingoes (fig. 5). Although we

411 observe a small enrichment in the rest of the categories, we hypothesize these results
412 may be due to the small sample size and low genetic diversity in K'gari dingoes compared
413 to mainland dingoes. To assess the effect of sample sizes on our results, we conducted
414 this analysis using the same number of samples for each dingo population and found that
415 results stayed very similar (Suppl. Fig. 5).

416 DISCUSSION

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

428 In large and outbred populations strongly deleterious recessive alleles are "hidden" from
429 selection in heterozygous form, making large populations more prone to experiencing
430 inbreeding depression after going through a harsh bottleneck (Robinson et al. 2018;
431 Kyriazis et al. 2021). On the other hand, small and isolated populations tend to have a
432 higher burden of deleterious variants as a result of inbreeding (Robinson et al. 2018;

433 Hedrick et al. 2019; Grossen et al. 2020; Khan et al. 2021; Stoffel et al. 2021). We observe
434 a similar trend in K'gari dingoes, finding higher levels of homozygosity in this population
435 compared to canids from large and stable populations, such as wolves and village dogs.

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

447 It has been observed that long-term small populations (i.e., populations that experienced
448 early bottlenecks and have maintained small population size following the bottlenecks)
449 can persist despite showing high levels of inbreeding by purging strongly recessive
450 deleterious variants (Xue et al. 2015; Benazzo et al. 2017; Robinson et al. 2018; Grossen
451 et al. 2020), whereas populations that have gone through multiple recent bottlenecks will
452 most likely experience inbreeding depression despite purging (Khan et al. 2021; Stoffel
453 et al. 2021). Previous studies have documented dingoes in K'gari have experienced at
454 least two bottlenecks in the form of large-scale culling events during the past 120 years
455 (Petrie 1995; Allen et al. 2015). Our assessment of purging in this population indicates

that while strongly deleterious alleles are likely being purged, there is little to no purging of mildly deleterious alleles in this population. This may have important long term consequences for the health of the K'gari dingoes that should be considered by conservation and management programs.

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

479 closely monitoring for signs of inbreeding depression and more research (e.g. population
480 genetic simulations) have been recommended under similar scenarios (Robinson et al.
481 2018; Kyriazis et al. 2021; Robinson et al. 2022) to explore the effectiveness of this
482 strategy, given that non-genetic factors (e.g. environmental stressors) may also play a
483 role in population persistence.

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

499 Although dingoes are protected on K'gari, they are euthanized due to injury, poor health,
500 or as a result of high-risk behavior towards humans. Moreover, QPWS have documented
501 conspecific killing (Behrendorff et al. 2018), vehicle strikes and snake bite, and young

502 dingoes may be susceptible to predation such as goannas, snakes, and birds of prey.
503 There is considerable debate about how the population should be managed, both in terms
504 of conservation and how to reduce risk to tourists visiting the island. This work helps
505 broaden our knowledge and understanding about the evolutionary history and genetic
506 health of K'gari dingoes in order to better inform conservation and public policies. Our
507 work, although limited by a small sample size and the sampling of several closely related
508 samples, indicates that the dingo population on K'gari is challenged by inbreeding as a
509 result of founder effects and successive bottlenecks. We find the first evidence that K'gari
510 dingoes may be purging strongly deleterious mutations, whilst accumulating a higher
511 genetic load of mild/slightly deleterious mutations. Further research is needed to
512 investigate the genetic mutation load of dingoes on K'gari using a larger sample size, as
513 well as biological monitoring of the population for symptoms of inbreeding depression
514 such as reduced fertility, increased stillbirths, and congenital birth defects. QPWS and
515 Butchulla Prescribed Body Corporates are left with the challenge of preserving dingoes
516 on K'gari, balancing the need to address human safety concerns and protecting the dingo
517 population's genetic health and viability into the future.

518

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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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825

826 **FIGURES**

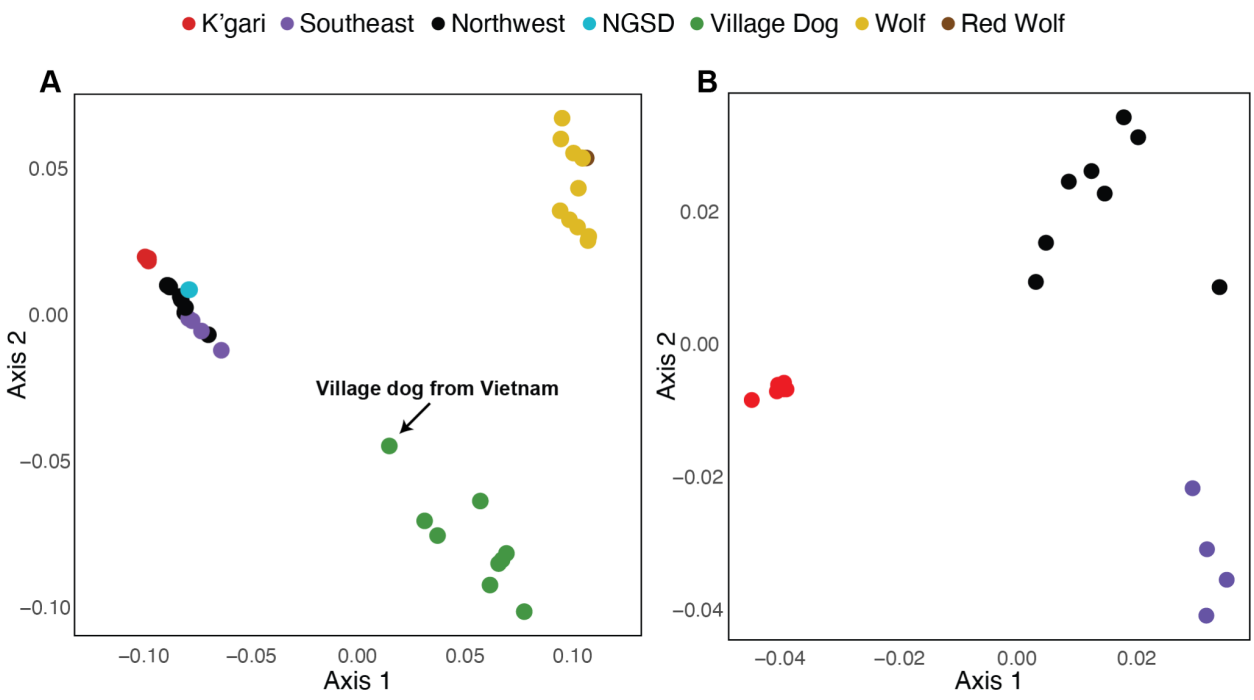

Figure 1. Reduced dimensional representation of sample clustering based on multidimensional scaling of the allele sharing dissimilarity matrix. First two major axes of variation plotted for (A) all 41 canids included in this study and (B) 18 dingo individuals.

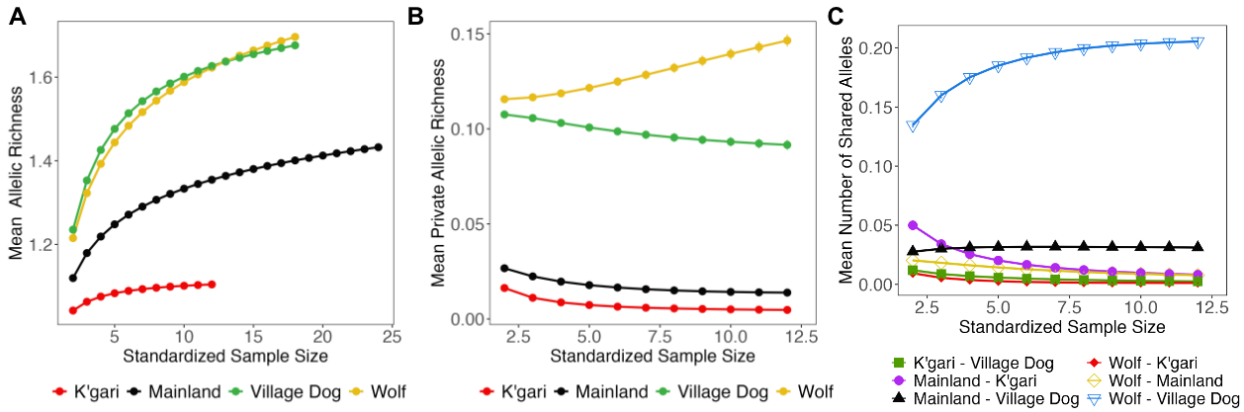

Figure 2. Genetic diversity estimates within and between canid populations. Error bars representing 95% confidence intervals for each line are too narrow to be visible. (A) Mean

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.

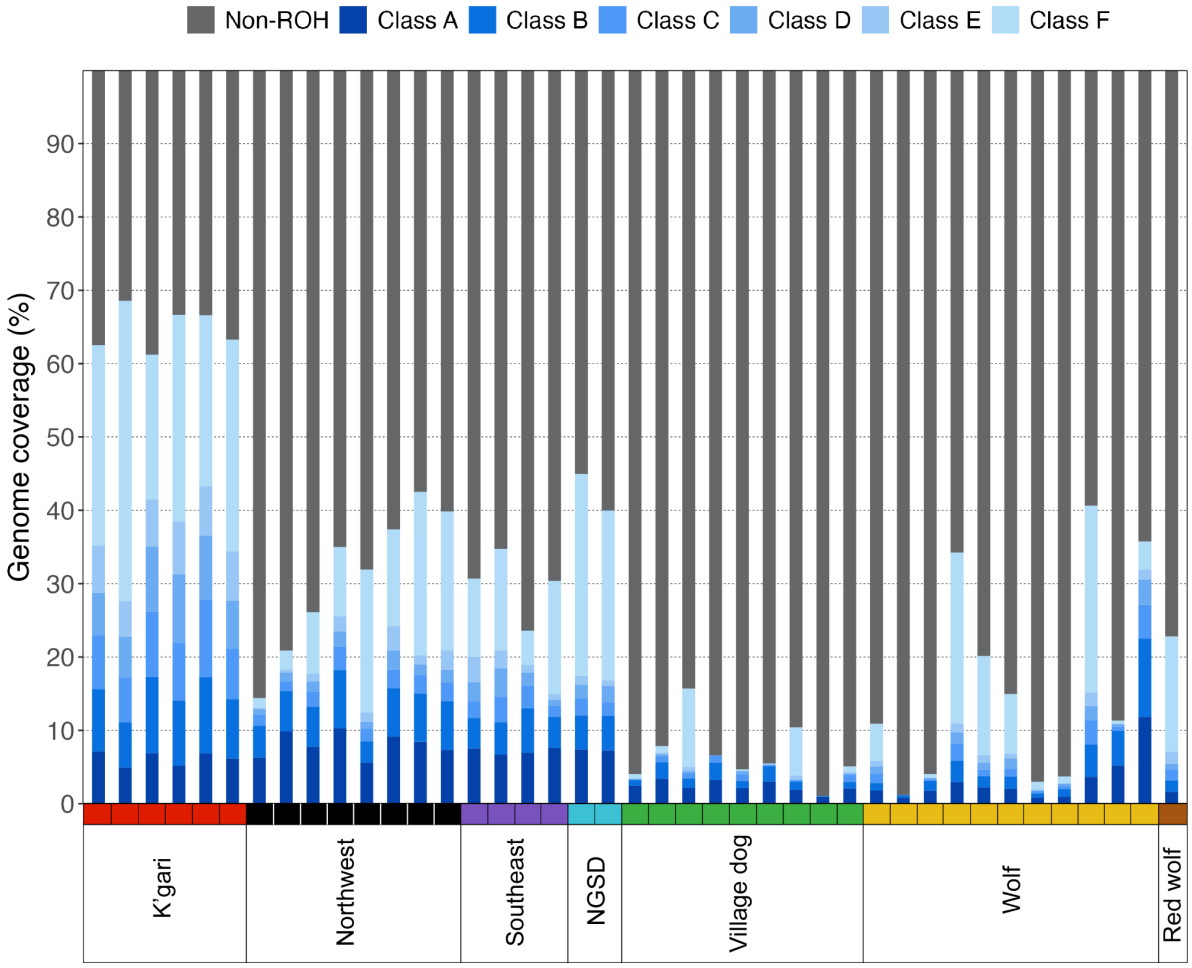

Figure 3. ROH coverage across individual genomes. The percentage of the genome covered by class A ROH (0.5-1 Mbp), class B ROH (1-2 Mbp), class C ROH (2-3 Mbp), class D ROH (3-4 Mbp), class E ROH (4-5 Mbp), and class F ROH (>5 Mbp) is shown on the Y-axis for each of the 41 canids included in this study.

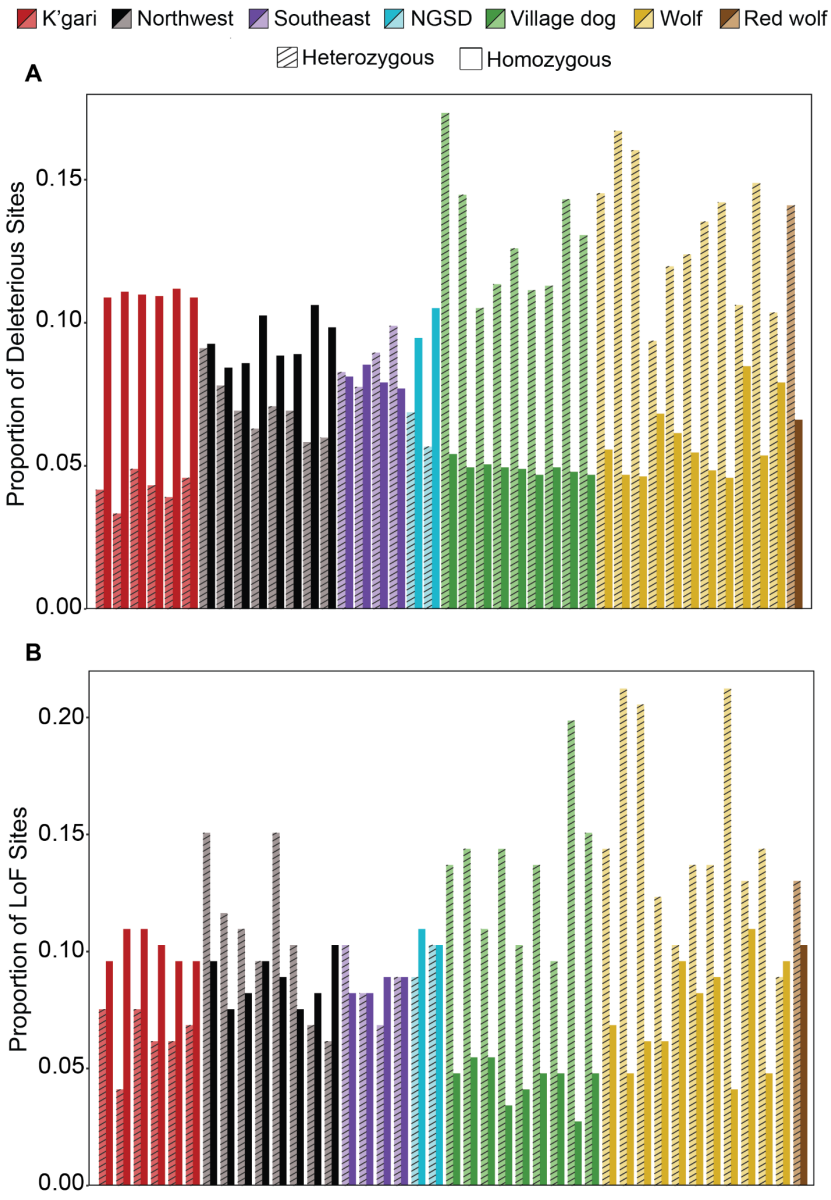

Figure 4. Predicted damaging alleles. (A) Predicted deleterious variants. (B) Loss-of-Function (LoF) variants. For each category solid bars show the proportion of homozygous sites, and striped bars show the proportion of heterozygous sites for each canid individual of each population.

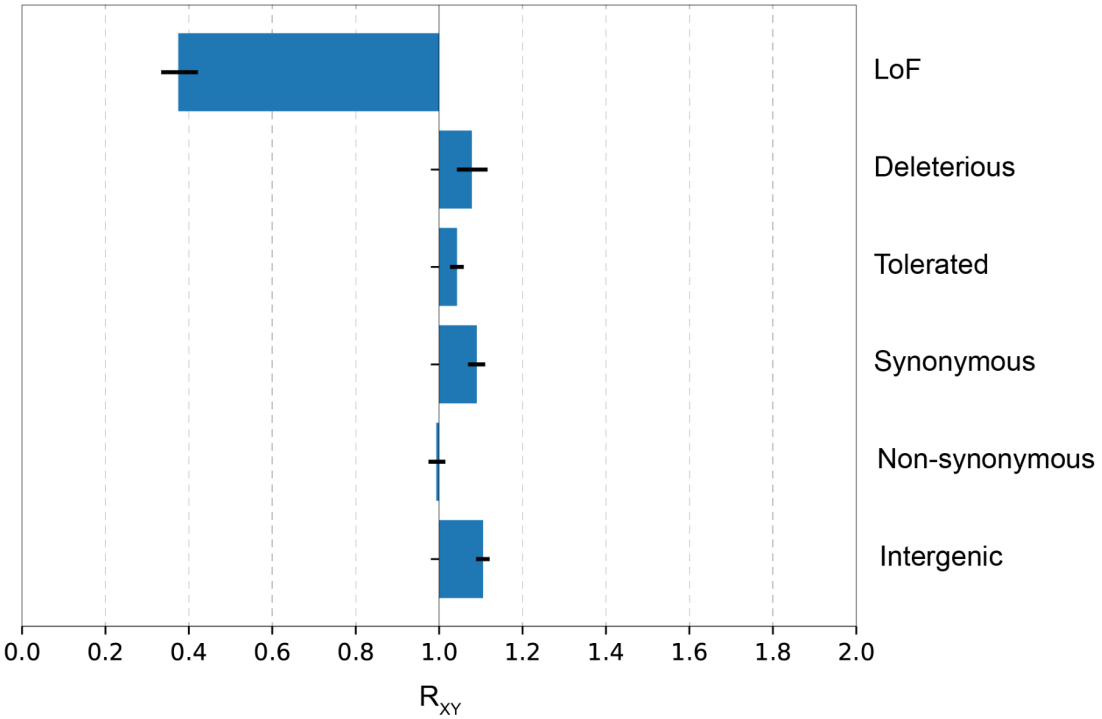

Figure 5. Kinship-weighted R_{xy} estimates for K'gari and mainland dingoes. Estimates represent the relative excess number of derived alleles for each category in K'gari dingoes (population X) relative to mainland dingoes (population Y). Error bars represent the minimum and maximum values for the 95%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