Genomic evidence for inbreeding depression and purging of deleterious genetic variation in Indian tigers

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Abstract

Increasing habitat fragmentation leads to wild populations becoming small, isolated, and threatened by inbreeding depression. However, small populations may be able to purge recessive deleterious alleles as they become expressed in homozygotes, thus reducing inbreeding depression and increasing population viability. We used whole genomes sequencing from 57 tigers to estimate individual inbreeding and mutation loads in a small-isolated, and two large-connected populations in India. As expected, the small-isolated population had substantially higher average genomic inbreeding (F_{ROH}=0.57) than the large-connected (F_{ROH}=0.35 and F_{ROH}=0.46) populations. The small-isolated population had the lowest loss-of-function mutation load, likely due to purging of highly deleterious recessive mutations. The large populations had lower missense mutation loads than the small-isolated population, but were not identical, possibly due to different demographic histories. While the number of the loss-of-function alleles in the small-isolated population was lower, these alleles were at high frequencies and homozygosity than in the large populations. Together, our data and analyses provide evidence of (a) high mutation load; (b) purging and (c) the highest predicted inbreeding depression, despite purging, in the small-isolated population. Frequency distributions of damaging and neutral alleles uncover genomic evidence that purifying selection has removed part of the mutation load across Indian tiger populations. These results provide genomic evidence for purifying selection in both small and large populations, but also suggest that the remaining deleterious alleles may have inbreeding associated fitness costs. We suggest that genetic rescue from sources selected based on genome-wide differentiation should offset any possible impacts of inbreeding depression.

Significance statement

Habitat fragmentation is sequestering species into small and isolated populations with high chances of extinction. But are small and isolated populations at risk of extinction from inbreeding depression, or does inbreeding and purging of deleterious alleles reduce such threat? Using whole genomes from several wild Indian tiger populations, we provide evidence supporting purging of highly deleterious variants in a small isolated population. However, our analyses also indicate that the remaining highly deleterious alleles are at high frequencies, suggesting continued inbreeding depression despite some successful purging. We discuss the implications of our results for conservation, including possible genomics-informed genetic rescue strategies.

Introduction

A large proportion of the earth's biodiversity persists in small and isolated populations in today's anthropogenically modified world[1]. Such populations may suffer from decreased genetic variation and increased inbreeding[2] which together lead to decreased fitness and increased extinction risk[3]. Several theoretical[4,5,6,7] experimental[8] and empirical studies (for example 9) reveal that species surviving in small and isolated populations are at the greatest risk of extinction.

While species exist in nature along a continuum from small to large populations with different levels of isolation, populations of endangered species often tend to be small and isolated. African wild dog, Ethiopian wolf, and great Indian bustard are examples of species where all populations are small and isolated[10,11,12]. The 'small population paradigm' of conservation biology suggests that such smaller and more isolated populations are at a higher risk of extinction due to inbreeding depression and demographic stochasticity[13,14,15].

Inbred individuals express deleterious, partially recessive alleles that are inherited identical-by-descent (IBD) from related parents, leading to inbreeding depression[16]. Such inbreeding depression can reduce the average fitness of a population,

eventually leading to reduced population size, and possibly extinction[17]. A commonly adopted strategy to conserve inbred populations is genetic rescue[18], which aims to increase average fitness by decreasing the frequency of deleterious mutations and increase heterozygosity at loci harboring deleterious alleles, via translocations of individuals from genetically differentiated populations. A meta-analysis of empirical data from wild populations showed broadly consistent positive effects of genetic rescue on fitness[15,19].

Population genetic theory[20,21,22] predicts that purifying selection can reduce inbreeding depression by purging deleterious alleles from inbred populations in the absence of immigration. Whether isolated populations are likely to purge a substantial fraction of the mutation load has been of longstanding interest in evolutionary biology and conservation. Early empirical data from pedigreed captive populations suggested that purging was either absent, or resulted only in slight decreases in inbreeding depression (e.g., 23,24). However, several experimental studies based on model organisms reveal substantial purging and significant reduction in inbreeding depression in small populations25,26,27. Recent molecular and population genetic studies have found genomic evidence for purging in wild populations (e.g., 28,29,30). Despite broad empirical support for the efficacy of genetic rescue[15,19], genomic evidence for purging and the long term persistence of some small isolated populations have been cited to question the small population paradigm, and to argue that standard genetic rescue practices are likely to be counterproductive[29,31,32,33]. Whether purging removes enough deleterious alleles to improve the viability of small, isolated populations (contradicting the small population paradigm) remains an open question. We address this question by contrasting genomic inbreeding and mutation load in small isolated versus large-connected populations of wild tigers.

We use Bengal tigers (*Panthera tigris tigris*) from India as a model to investigate levels of inbreeding and relative mutation loads in small-isolated and large-connected populations, and examine the potential for genetic rescue. Tigers are large, endangered carnivores, but Bengal tigers have high genetic variation compared to other sub-species, with some sub-populations showing high inbreeding

105 indicating isolation[34]. All Bengal tiger populations have been through historic
106 bottlenecks, but inbreeding and genetic variation vary among populations. Also,
107 some populations are relatively large and connected while others are small and
108 isolated from both genetic[35] and demographic perspectives[36,37], making them an
109 ideal system to investigate inbreeding, mutation load and possible genetic rescue
110 strategies.

111 We use genomic data to measure the impact of historically declining population sizes
112 and connectivity on inbreeding and mutation load in three wild Bengal tiger
113 (*Panthera tigris tigris*), populations that are small-isolated (SI) and large-connected.
114 The small-isolated population is from north-western India and the large-connected
115 populations are from south India (s-LC) and central India (c-LC). We expected large-
116 connected populations to be the least inbred and to have the lowest mutation load.
117 Alternatively, purging could result in lower mutation load in this small-isolated
118 population. We also explore strategies for genetic rescue that might effectively
119 decrease inbreeding depression. For tigers, we specifically suggest strategies for
120 identifying populations that may benefit from genetic rescue, and how such
121 strategies may be effectively implemented.

122 **Results and Discussion**

123 *Genomic estimates of inbreeding*

124 Tigers exist as several genetic population clusters within India with the total
125 population size of these clusters ranging from 60 to 1000 individuals. Each genetic
126 cluster consists of several protected areas, with varying connectivity with other such
127 protected areas and clusters[35,36,38]. For the purpose of this paper, we defined
128 populations, and whether they are 'isolated' or 'connected' based on population
129 genetic and gene flow analyses using markers across the genome[34,35,39]. Here, the
130 'large' populations currently have hundreds of tigers (minimum 300 tigers), while
131 'small' populations currently have below 100 tigers[36]. While both large populations
132 are part of connected landscapes, they do not have identical histories of connectivity.
133 The southern large-connected population is disconnected from other tiger population

genetic clusters in India (e.g. from Central India and Northwest), while central large-connected population (Central Indian landscape) was connected by gene flow to other tiger genetic clusters until recently (see 39,35). SFS-based demographic history models in Armstrong et al.[34] suggest that most Indian tigers diverged from each other recently. Increased agriculture, bounty hunting and illegal poaching, habitat loss and fragmentation together have led to local extinctions of tiger populations[40,41]. The small-isolated population we studied here has less than one hundred individuals, but was connected (until very recently) to the central large-connected population[42] and potentially to the now extinct tiger population in Afghanistan[43].

We quantify inbreeding by identifying long stretches of the genome that are homozygous and identical by descent (i.e., runs-of-homozygosity, ROH). The summed length of ROH divided by total autosomal size is an accurate genomic measure of inbreeding (F_{ROH}) (e.g., 44). We found that the mean F_{ROH}, measured using ROH longer than 100Kb ($F_{ROH>100Kb}$), 1Mb ($F_{ROH>1Mb}$) and 5Mb ($F_{ROH>5Mb}$) of all individuals in our dataset (regardless of population) was above 0.48, 0.31 and 0.23 respectively. Tigers from the small-isolated population were most inbred with an average $F_{ROH>100Kb}$ of 0.57, while average inbreeding due to recent ancestors (on average up to 5 generations ago, contingent on assumed recombination rate from domestic cat[45]) was above $F_{ROH>5Mb}$= 0.37 (Figure 1a). The F_{ROH} was proportional to effective population size (N_e), with the small isolated population having lower N_e at all time intervals compared to both large and connected populations. This level of inbreeding is empirically associated with high risk of extinction[46,47,48]. However, shorter ROH could be due to shared demographic history of bottlenecks (e.g. F_{ROH}=100kb) and inbreeding depression may be observed at higher F values for large carnivores[49]. Pedigree based studies[50] have reported that an inbreeding coefficient of 0.47 is lethal for tigers.

We show that inbreeding was low in the large-connected populations compared to the small-isolated population (Figure 1a,b). We compared our estimates for zoo individuals with their known pedigree values to authenticate these results and found that estimates of inbreeding indeed correspond with pedigree values[51]

165 (Supplementary Table 1). Overall, the long ROH of ≥10Mb, signifying inbreeding
166 due to ancestors as recent as 3 generations, are rare in large populations but
167 frequent in the small-isolated population. The mean inbreeding due to recent
168 ancestors in the small-isolated population ($F_{ROH>10Mb}$) was above 0.28, which could
169 lead to several negative effects[46,48]. We found that the large connected populations
170 showed inbreeding due to recent ancestors (on average up to 5 generations ago) of
171 less than $F_{ROH>5Mb}$ = 0.1. The large southern population has more inbred individuals
172 than the large central population, potentially due to its geographical placement,
173 historic size (Figure 1d) and connectivity[35,36,39]. Overall, although the theoretical
174 expectation is that populations with N_e < 1,000 are unlikely to persist[7,30,52,53], this
175 result indicates that a population with a census size of ~600 tigers may be
176 sufficiently large to buffer short term inbreeding effects with some geneflow. The long
177 term viability of these populations remains to be tested. While there are instances of
178 small tiger (and other carnivore) populations persisting[36,37], this could possibly be
179 due to their ability to purge some deleterious alleles. This implies that other large
180 felid species, like lions[54], snow leopards[55,56] and jaguars[57] that often persist in
181 population sizes close to 600 may not necessarily experience the detrimental effects
182 of inbreeding depression over the short term. We caution that this is likely to depend
183 on founder effects and chance variation in the mutation load among populations (but
184 see 30), and does not address the likelihood for fitness decline over the long term[7].

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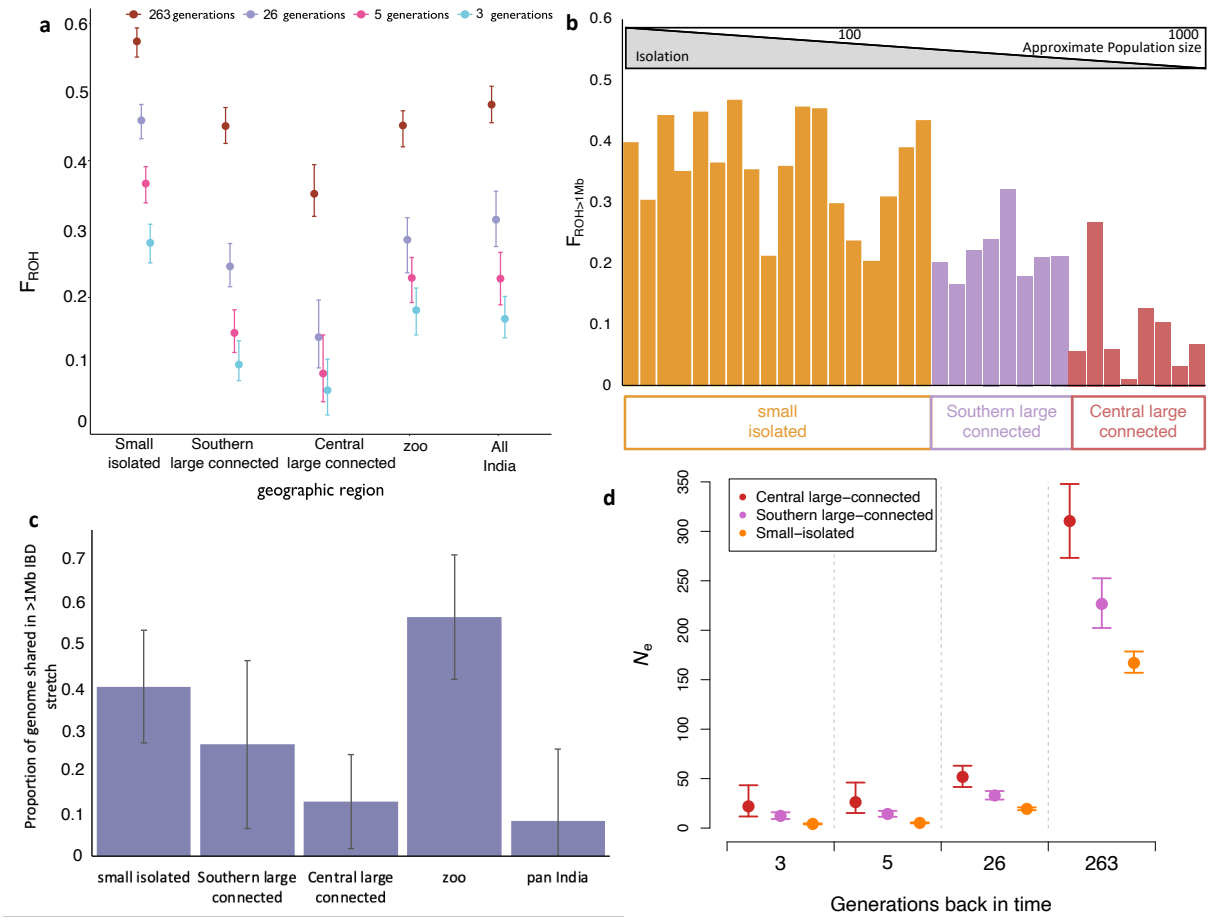

Figure 1: Inbreeding and shared ancestry in Indian tigers. Here we show cumulative inbreeding due to parental shared ancestry due to ancestors at different historical time ranges as estimated by different lengths of ROH, that is, ROH ≥ 10 Mb indicate shared ancestry on average up to 3 generations ago, 5 Mb stretches indicate on average up to 5 generations, 1 Mb stretches indicate on average up to 26 generations and 0.1Mb stretches indicate on average up to 263 generations ago. The figure shows cumulative inbreeding in each population due to shared ancestry between parents at each time range. Error bars are 95% CI. (a), inbreeding in individuals due to shared ancestry 26 generation ago (b), the proportion of the genome shared in in 1 Mb or longer tracts between pairs of individual from a population of individuals belonging to central large connected, southern large connected and small isolated populations. Pan India comparisons in (c) include all pairwise comparisons of wild individuals. Error bars are standard errors. N_e over time as estimated from F_{ROH} for the different populations. Error bars are standard deviations (d).

202 In addition to having the highest F_{ROH}, we found that pairs of individuals from the
203 small-isolated population also shared large tracts of genome that were IBD (Figure
204 1c). This could be a worrying sign for its long-term future as the pairwise IBD sharing
205 translates into inbreeding in the offspring. On average, pairs of individuals from the
206 small-isolated population shared about 40% of their genome (as more than 1Mb long
207 IBD stretches), while pairs from the southern large-connected and the central large-
208 connected populations share about 25% and 15% respectively, slightly higher than
209 the corresponding mean F_{ROH} values (Figure 1a). As with inbreeding in offspring,
210 pairwise IBD sharing is expected to be influenced by founding bottlenecks in addition
211 to recent small N_e (Figure 1d). The small-isolated population had the lowest
212 mitochondrial haplotype diversity and the lowest number of mitochondrial haplotypes
213 (Supplementary Figure 1). This lack of diverse lineages, large tracts of shared IBD
214 genome between individuals, and the recent small population size may therefore
215 require continued immigration of individuals to sustain genetic variation[58], or in the
216 absence of natural immigration, may require genetic rescue.

217 *Mutation loads in small-isolated and large-connected tiger populations*

218 We evaluated the relationships between F_{ROH} and the frequency and genomic
219 distribution of putatively deleterious genotypes identified using the Ensembl Variant
220 Effect Predictor[59] (VEP). Under the assumption that most deleterious alleles are
221 partially recessive[28,60,61], the number of homozygous damaging alleles is likely to be
222 informative of the fitness cost of individual inbreeding. First, we found that the
223 number of homozygous putatively damaging alleles (loss-of-function (LOF) and
224 missense alleles together) was proportional to F_{ROH} in all populations. The number of
225 putatively damaging homozygotes increased from <1500 for individuals with $F_{ROH} \approx$
226 0.3 to approximately 2200 for individuals with $F_{ROH} \approx 0.6$ ($P < 0.01$ for each
227 population, linear regressions; Figure 2). This genomic evidence suggests a fitness
228 cost associated with increased inbreeding. Additionally, the burden of homozygous
229 putatively damaging mutations was substantially larger on average in the small-
230 isolated population (mean ≈ 2,000 per individual) than in the central large-connected
231 population (mean < 1,600 per individual, $P < 0.0001$ randomization test) or the
232 southern large-connected population (mean > 1,800 per individual, $P = 0.0025$,

233 randomization test, Figure 2). This is likely due to higher N_e in the central large-
234 connected population as compared to the southern large-connected population
235 (Figure 1d).

236 We further investigated differential mutation load between populations for LOF and
237 missense mutations identified with VEP, and the R_{XY} method[28,62] to estimate relative
238 excess of number of putatively deleterious alleles in one population compared to
239 another. R_{XY} compares the number of derived alleles of a particular category (LOF,
240 missense, or putatively neutral) found in one population relative to the other. If R_{XY} is
241 one, both populations X and Y have the same load of derived alleles, whereas if R_{XY}
242 > 1 then population X has more derived alleles than Y and vice versa if $R_{XY} < 1$ [28,62].
243 As above, we found a higher load associated with missense mutations in the small-
244 isolated population (Figure 2c). This is likely explained by more efficient purifying
245 selection against missense mutations of relatively small effect, which are typically
246 only slightly recessive[61], due to larger N_e in the large populations than in the small
247 population. We also found a lower load of LOF mutations in the small-isolated
248 population compared to the large populations (Figure 2c). This suggests that higher
249 inbreeding in the small-isolated population facilitated purging of relatively large-effect
250 LOF mutations – which are more likely to be highly recessive than small-effect
251 deleterious mutations[61,63]. Together, these different approaches suggest a higher
252 putatively damaging allele load in small isolated population coupled with purging of
253 loss-of-function alleles in the small isolated (and inbred) tiger population.

254 Despite this evidence for purging of LOF alleles in the small-isolated population, it
255 appears that high inbreeding lead to individuals in this population having a higher
256 number of homozygous LOF alleles on average (N=250) compared to the southern
257 large-connected (N=238) and central large-connected (N=218) populations (Figure
258 2b). High homozygosity for remaining LOF alleles suggests that the fitness cost due
259 to LOF mutations may be higher in the small-isolated population compared to the
260 large populations despite partial purging. Importantly, while it is impossible to know
261 the realized fitness effects of homozygosity for LOF and missense mutations, this
262 analysis suggests that purging was not sufficient to result in a smaller fitness cost of
263 inbreeding in the small-isolated population compared to the larger populations. We

observe similar results when we used a Genomic Evolutionary Rate Profiling (GERP)[65] analysis to identify potentially highly deleterious alleles by keeping loci with GERP scores in the top 0.1 percent (Supplementary Figure 3). For this analysis, and those below, it important to recognize that our measure of mutation load is not a direct measure of fitness. Rather, our inferences rest on the assumption that missense and LOF mutations, and mutations at conserved sites are deleterious on average, which is well supported empirically[65,66]. Definitively determining whether the bottlenecks faced by these populations[40,41] and the additional bottleneck faced by north-west Indian population three generations ago in 2004[67] purged part of the mutation load would require historical genetic data to quantify pre- versus post-bottleneck mutation loads. Additionally, better estimates of very recent demographic history would help to predict the potential for purging during the bottlenecks.

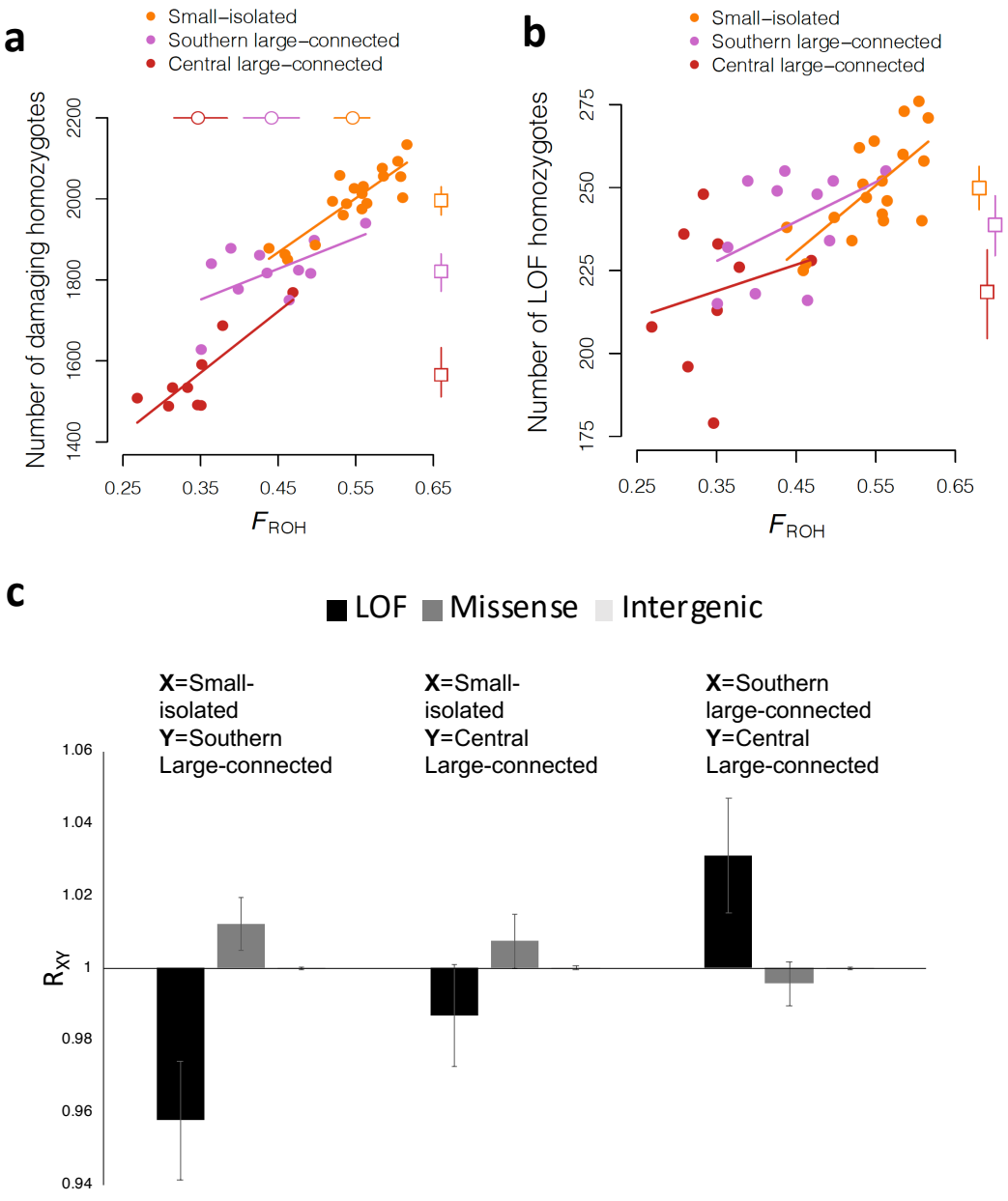

Figure 2 Relationships between inbreeding and number of homozygous damaging alleles (LOF plus missense) **(a),** and homozygous LOF mutations **(b)**, and relative mutation loads in populations of various sizes and levels of isolation **(c)**. Filled circles represent the values of F_{ROH} (x-axis) for different individuals, and the number of homozygous damaging mutations (y-axis), with each population represented by color as indicated in the legend. Open circles and squares represent the mean F_{ROH} and mean number of homozygous damaging mutations, respectively, for each population, with the error bars representing 95% percentile bootstrap confidence intervals. R_{XY} indicates excess of deleterious alleles in population X relative to Y. All

287 populations had similar numbers of neutral (intergenic) mutations, and error bars are
288 standard errors from jacknife in **(c)**.

289 Annotated sets of deleterious variants are rarely available for endangered species.
290 We compared our missense mutations to annotated sets of domestic cat mutations
291 (Supplementary Table 2). The missense mutations we identified mapped to genes
292 responsible for disease states such as hypertrophic cardiomyopathy, progressive
293 retinal atrophy, polycystic kidney disease, cystinuria, gangliosidosis, hyperoxaluria,
294 hypothyroidism, mucolipidosis and mucopolysaccharidosis. Further studies are
295 needed to functionally validate the effect of these mutations in tigers. Studies on
296 inbred/small populations of Asiatic lions revealed several abnormalities[68], and
297 genomic studies here might provide further insights on deleterious mutations in large
298 cats. In the small-isolated population at least one tiger has a potential eye condition
299 (Supplementary Figure 2), although no skeletal defects were observed in an earlier
300 study[69]. Additional quantitative genetic studies on inbred zoo individuals can aid in
301 understanding the possible phenotypic effects of inbreeding and inbreeding
302 depression for endangered species.

303 Site-frequency spectra (SFS) revealed that nearly 14% of both putatively damaging
304 and neutral derived alleles were fixed in the small-isolated population (Figure 3). A
305 smaller proportion of derived alleles (both damaging and neutral) were fixed in the
306 large populations (< 0.06). However, there were slightly more fixed derived alleles in
307 the southern large connected population than in the central large-connected
308 population. The higher abundance of fixed derived alleles (both damaging and
309 neutral) in the small-isolated population, and to a lesser extent in the southern large-
310 connected population, suggests that genetic drift (e.g., due to historical population
311 bottlenecks and founder effects) drove previously rare alleles to fixation in these
312 populations (Figure 3)[70]. The fixation of deleterious alleles is consistent with
313 theoretical predictions for very small populations[4,7] and empirical observations from
314 wolves[32], Apennine brown bears[71] and other species[31]. In the Isle Royale wolves,
315 high frequency deleterious alleles were consistent with frequent bone deformities in
316 the population[32,72]. The large fraction of fixed putatively damaging mutations in the
317 small isolated population suggests that partial purging of the mutation load due to

318 LOF mutations (Figure 2c) was not sufficient to erase a substantial fraction of the
319 mutation load in this population. While the SFS was essentially flat for polymorphic
320 loci (i.e., excluding loci with derived allele frequencies of 0 or 1) in the small isolated
321 population (as expected following a population bottleneck), there was a larger
322 fraction of rare derived alleles in the large populations, as expected for large
323 populations at mutation drift equilibrium[70]. A larger proportion of putatively damaging
324 than neutral alleles were at or near fixation in each of the large populations, and
325 across all of India (Figure 3), possibly due to positive selection driving selective
326 sweeps on a small fraction of non-synonymous mutations. The hump-shaped SFS
327 are likely the result of a complex demographic history for Indian tigers, including
328 strong population structure, and historical population bottlenecks[70,73,74].

329 Lastly, the frequency distributions of putatively deleterious and neutral derived alleles
330 reveals signatures of purging via natural selection. Some previously rare, deleterious
331 alleles are expected to be removed from a population by random genetic drift alone,
332 during periods of small N_e (e.g., population bottlenecks or founding events). Effective
333 natural selection through purging is expected to eliminate deleterious derived alleles
334 more frequently than neutral alleles, or to keep them at lower frequencies than
335 neutral derived alleles. We expected putatively deleterious derived alleles to either
336 be absent, or to be less frequent (on average) than putatively neutral derived alleles
337 if purging has occurred in our study populations. This prediction was supported via a
338 randomization test in each population and for all populations combined (P < 2e-4, 2-
339 sided randomization test). This suggests that purifying selection has successfully
340 removed a small fraction of the mutation load in each of the three study populations
341 and across India. The higher proportion of fixed and very high frequency derived
342 putatively damaging alleles in the large populations and across Pan India may be
343 caused by historical selective sweeps on a small fraction of positively selected
344 nonsynonymous mutations, the details of which we leave to future research.

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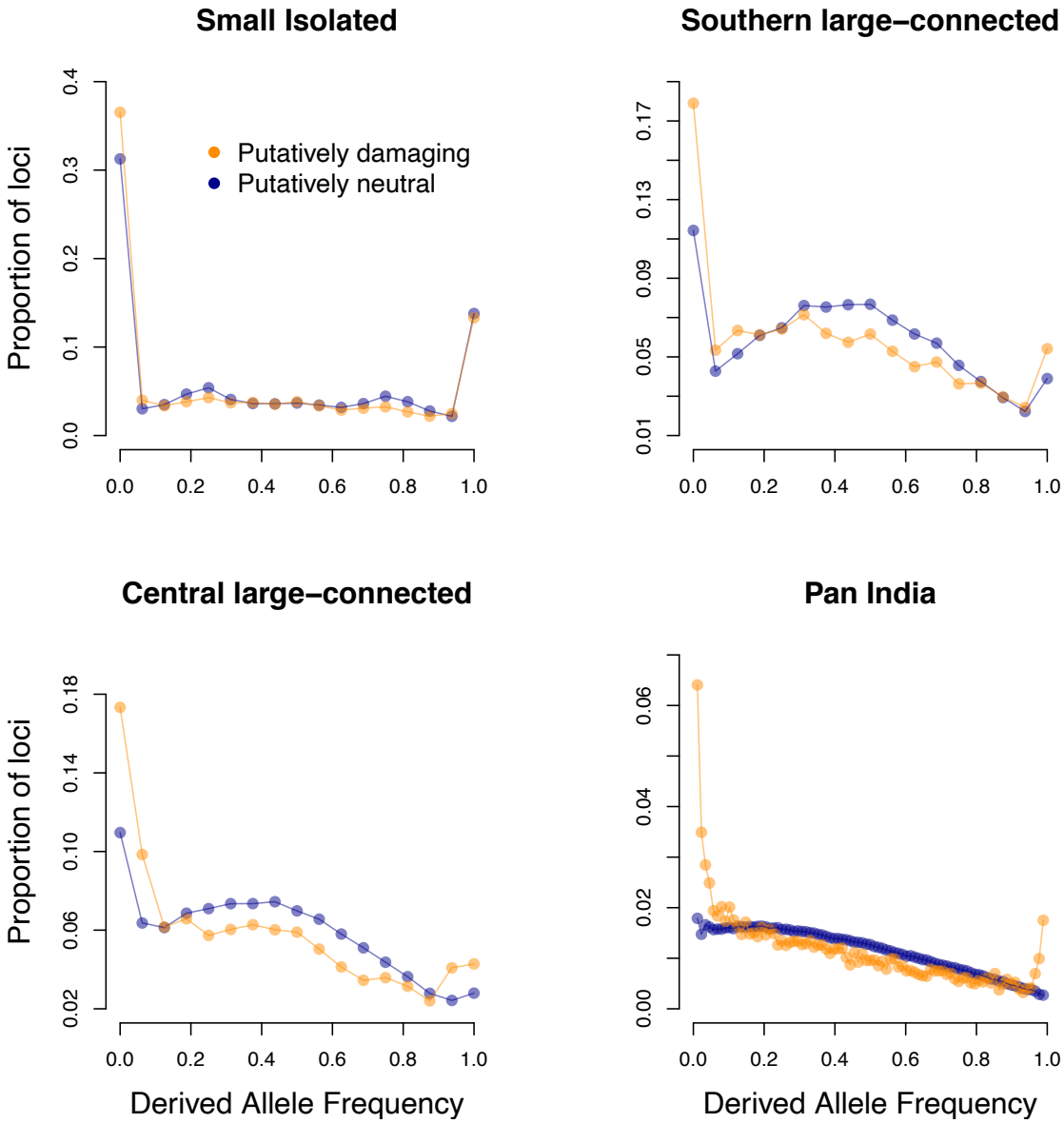

346

347 **Figure 3.** Site frequency spectrum for damaging (missense and LOF mutations
348 combined, orange) and neutral mutations (blue) for each population. The proportion
349 of loci (y-axis) is shown for each possible derived allele frequency (x-axis). Sample
350 sizes were equalized among the three populations by randomly subsampling 16 non-
351 missing alleles for each locus in each of the three populations. Fixed (frequency=1)
352 and missing (frequency=0) alleles are included in the SFS for the three individual
353 populations. We subsampled 47 non-missing alleles at each locus to calculate the
354 SFS for pan India to account for missing genotypes.

355 Interestingly, both inbreeding and mutation load for the southern large connected
356 population (from Western ghats in India) are higher than the central large connected
357 population (from Central India, Figures 1 and 2). This could reflect the central
358 location of this population (see 35,38,39). Additionally, the central large connected
359 population may have had higher historical connectivity (with other population
360 clusters), a two dimensional network of local populations (versus a linear array in
361 southern large connected populations) and other ecological factors that allow higher
362 population densities to be achieved in all habitats of central large connected
363 population compared to southern large connected populations.

364 *Genetic rescue strategies*

365 Our data suggest that genomic inbreeding (F_{ROH} up to 0.6 in the small isolated
366 population) is comparable to populations experiencing inbreeding depression (e.g.
367 Florida puma, F_{ROH} up to 0.6[75] and Isle Royale wolves, F_{ROH} between 0.1 and 0.5[32]).
368 While we also demonstrate the effects of purifying selection, homozygous LOF
369 alleles are more frequent, and mildly deleterious missense mutations are in excess
370 in the small isolated population.

371 Gene flow is the proposed strategy to sustain small and isolated
372 populations[18]. Geneflow that results in masking of deleterious alleles responsible for
373 genetic load, leading to increased population growth rate is genetic rescue.
374 Evolutionary rescue is geneflow that increases genetic variation[76] and thus adaptive
375 potential. High F_{ST} at loci with high frequency damaging alleles in the receiving
376 population (frequency > 0.9) should be indicative of good source populations for
377 genetic rescue. In practice, for both evolutionary rescue and genetic rescue, source
378 populations are identified as those that have high genome-wide genetic
379 differentiation with the receiving population (to maximize the heterozygosity of
380 admixed offspring (reviewed in 19)). Geneflow from populations with high genome-
381 wide F_{ST} in theory should result in masking deleterious alleles, allowing both genetic
382 rescue and evolutionary rescue. However, the smaller number of loci with high-
383 frequency damaging alleles compared to all polymorphic loci could lead to
384 discordance between these two proxies for genetic and evolutionary rescue (i.e.,

genetic differentiation at loci with putatively damaging alleles may not be a good predictor of genome-wide genetic differentiation).

We investigated whether evolutionary rescue is a good proxy for genetic rescue. We propose that certain source populations may be ideal if they result in masking of damaging alleles and increased genome-wide heterozygosity (top right quadrant of Figure 4a). Alternatively, other source populations (bottom right quadrant) will increase heterozygosity but not mask damaging alleles, while those in the top left quadrant mask damaging alleles but may not increase heterozygosity substantially genome-wide (Figure 4a). Our data and estimates reveal a strong and positive correlation between the two proxies (Figure 4b, adjusted r^2 = 0.91), re-enforcing that selecting source populations based on high genome wide F_{ST} would result in both genetic and evolutionary rescue. For the small isolated population, our data suggest that Kaziranga (proportion of damaging loci rescued = 0.74; Genome-wide F_{ST} = 0.26, damaging loci F_{ST} =0.26) is the best source population, while ecologically more similar populations like Kanha (proportion of damaging loci rescued = 0.69; Genome-wide F_{st} = 0.22; damaging loci F_{ST} =0.23) and Corbett (proportion of damaging loci rescued = 0.65; Genome-wide F_{st} = 0.26; damaging loci F_{ST} =0.28) may be more practical, and almost as effective at genetic rescue. For the large isolated population in Southern India, both proxies are lower on an average (average proportion of damaging loci rescued = 0.65; average genome-wide F_{ST} = 0.195, average damaging allele F_{ST} = 0.21, across source populations). Note that high genome-wide F_{ST} between a donor and recipient population could also be due to intense bottlenecks and resulting drift in endangered species, and such population pairs may not result in effective genetic rescue. We caution that these inferences are subject to assumed fitness effects of damaging alleles. Most genome-wide predictions of deleterious alleles in wild species await annotation with phenotypic data[77], such a strategy could be implemented when the loci affecting fitness are identified as in the case of Tasmaninan devils (*Sarcophilus harrisii*[78]) and Soay sheep[79].

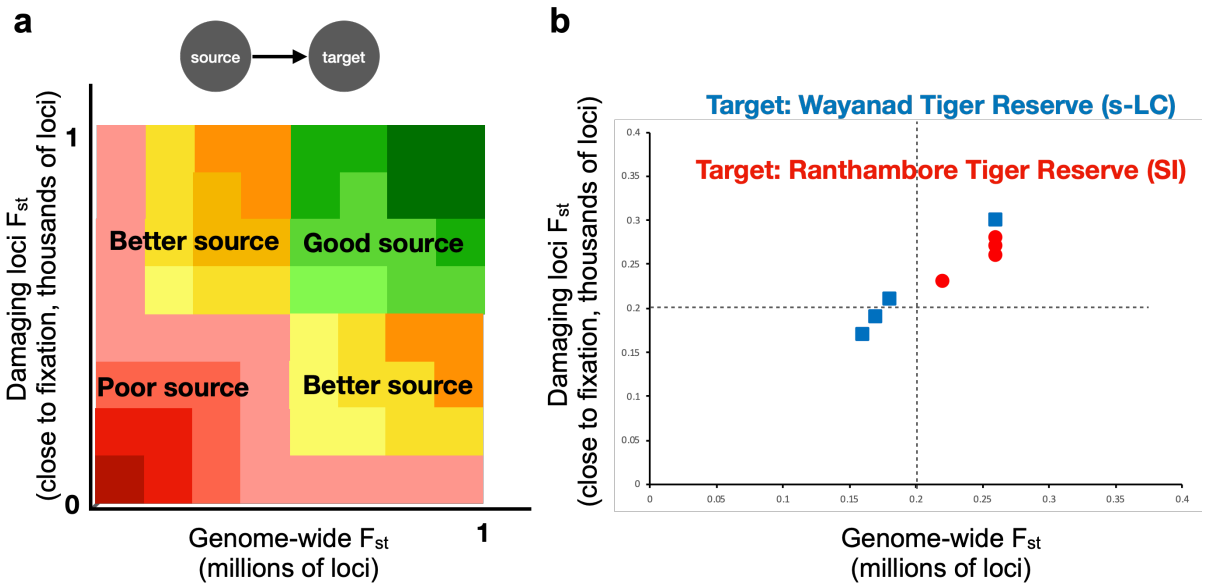

Figure 4. (a) A schematic showing the possible distribution of genome-wide F_{ST} and (b) the observed values for our data, assuming two target populations, Ranthambore Tiger Reserve (in red circles, the small isolated population) and Wayanad Tiger Reserve (blue squares, a protected area within the Southern large connected population). F_{ST} on the y-axis is for loci where the putatively damaging allele is at a frequency >0.9 in the target population, and for all loci across the genome on the x-axis.

Whether there is ever a substantial advantage to selecting source populations for genetic rescue attempts based on differentiation for putatively damaging loci (versus across the whole genome) remains to be tested empirically. Genome wide surveys would help in identifying source populations that best mask damaging alleles and increase genome-wide heterozygosity; however there are other considerations (as highlighted in 33) that could impact selection of source populations. A denser sampling of tigers across the Indian tiger range is necessary to predict best rescue individuals for particular target populations. Such dense sampling of genomes can benefit from sequencing of non-invasive samples since acquiring blood samples from endangered species can be difficult[80]. Methods involving sequencing genomes from shed hair[81] or fecal samples[82] will be useful in creating such translocation networks and monitoring the populations for gene flow.

Implications for conservation of endangered species

We find that small and isolated populations, in this case tigers of Ranthambore Tiger Reserve, have high inbreeding arising from both recent ancestors and ancestors in deep history. Our analyses revealed a signature of purging for putatively large effect (LOF) deleterious alleles in this population. However, this small and isolated population also had the highest average number of homozygous damaging alleles of the three study populations, suggesting potential fitness cost of inbreeding in this population despite purging of some large effect mutations, and compared to the large and connected populations. Together, these results demonstrate that purging (as inferred from genomic signatures) does not eliminate all damaging alleles, and hence does not contradict the small population paradigm of conservation biology[19]. Careful predictions of putative phenotypic effects of the existing deleterious alleles at high frequencies might be instructive to understand future inbreeding depression, and these alleles could potentially be used as early warning signs for population decline. However, caution is warranted in this regard because it is still unclear how effectively molecular predictions (i.e., identification of loss of function or derived alleles at conserved sites) can translate into predictions of fitness differences among extant individuals and populations.

Historical isolation and bottlenecks due to overharvest appear to have a significant effect on missense (potentially mild effect) mutation load. Our analyses suggest that purging can also occur in large-connected populations in the context of endangered species (Figure 3). This could be because all tiger populations are in general small, and the range of population sizes is not very wide[36]. Continued isolation of these populations will increase mutation load in the future. Thus maximizing connectivity might be the best strategy to minimize extinction (from an ecological perspective), increase genome-wide genetic variation, and also minimize mutation load in the future. Maximizing connectivity in the wild would require increasing population abundance since large populations are sources of dispersing individuals, but also planning for habitat corridors, since demographics of tiger landscapes are dominated by extinction and recolonization dynamics (e.g. see 83, 84). Such strategies may be

466 relevant to several endangered species with wide ranges but fragmented
467 populations, like elephants, lions and wild dogs.

468 Critical to providing recommendations for management is understanding ongoing
469 evolutionary trajectories for endangered species. Given the large number of frequent
470 deleterious alleles in the small isolated population, management could aim to
471 decrease the number of close to fixation damaging alleles, potentially maximizing
472 mean absolute fitness. Our data reveal that source populations with high genome-
473 wide differentiation (with the target population) would adequately mask deleterious
474 alleles and increase heterozygosity. Gene flow from even the relatively proximate
475 large-connected population in Central India would decrease the frequency of
476 damaging alleles, and allow genetic rescue. We caution that such gene flow could
477 also introduce other damaging alleles (e.g. in wolves, see 85).

478 Overall, genomic data and analyses provide richer, more nuanced ways to address
479 inbreeding and genetic rescue of small and isolated populations of endangered
480 species (i.e., the small population paradigm in conservation). We demonstrate
481 effective purging of some loss-of-function alleles, but not mutations with smaller
482 effects, highlighting the need to characterize detrimental mutations and how they will
483 be addressed through genetic rescue in future conservation efforts. Conservation
484 management based on predictive models for fitness effects in the context of
485 observed mutations will aid long-term persistence of populations in the wild.

486 **Methods**

487 *Sample collection*

488 We collected tissue from tranquilized or dead tigers from several tiger reserves (14
489 protected areas across India); zoo individuals from Sagar et al.[51] were also analyzed
490 (Figure 5, Supplementary Table 1). Individuals belonged to one of five population
491 clusters: 1) **Small-isolated population** from north-western India (NW) (n=19) **-** we
492 obtained tissues from 15 NW individuals and used data from two NW individuals in
493 Armstrong et al.[34] and two NW individuals in Khan et al.[81]. Our NW samples consist

494 only of individuals that were born in Ranthambore Tiger Reserve (RTR) but could
495 have been translocated to other protected areas (for example Sariska Tiger
496 Reserve) as per forest department records. 2) **Central Large-connected**
497 **population** from central India (C) (n=9) **-** we obtained tissue from four Kanha Tiger
498 Reserve (KTR) individuals and used data from Armstrong et al.[34] for five other KTR
499 individuals. 3) **Southern Large-connected population** from southern India (n=11) **-**
500 we obtained tissue from two Wayanad Wildlife Sanctuary (WWS) individuals and two
501 Bandipur Tiger Reserve (BTR) individuals. We additionally used data from
502 Armstrong et al.[34] for five WWS individuals and Liu et. al.[86] for two Nagarhole Tiger
503 Reserve (NTR) individuals. Since WWS, BTR and NTR populations are effectively
504 contiguous, we refer to them together as Wayanad (WAY) tigers. 4) **Nandankanan**
505 **Zoo**, Odisha, India – Sagar et al.[51] obtained five blood samples from inbred
506 individuals from Nandankanan Zoo, and these genomes were used as a control to
507 test our methods. The pedigree based inbreeding coefficient of these individuals vary
508 from 0.21 (n=1), 0.26 (n=1) and 0.28 (n=3) (Supplementary Table 1). Such pedigree-
509 based estimates are expected to be an underestimate of the actual inbreeding due to
510 the low depth of pedigrees[44]. 5) **Other protected areas -** we also sampled one
511 individual tiger from Lalgarh Division Forest, two individuals from Corbett Tiger
512 Reserve (COR), one from Chitwan National Park, four from Kaziranga Tiger
513 Reserve, one from Periyar Tiger Reserve, one from Bor Tiger Reserve, one from
514 Chandrapur district in Maharashtra and two from Sundarban Tiger Reserve. These
515 individuals combined with others are used for drawing pan-India inferences. The
516 sampling locations are depicted in Figure 6.

517

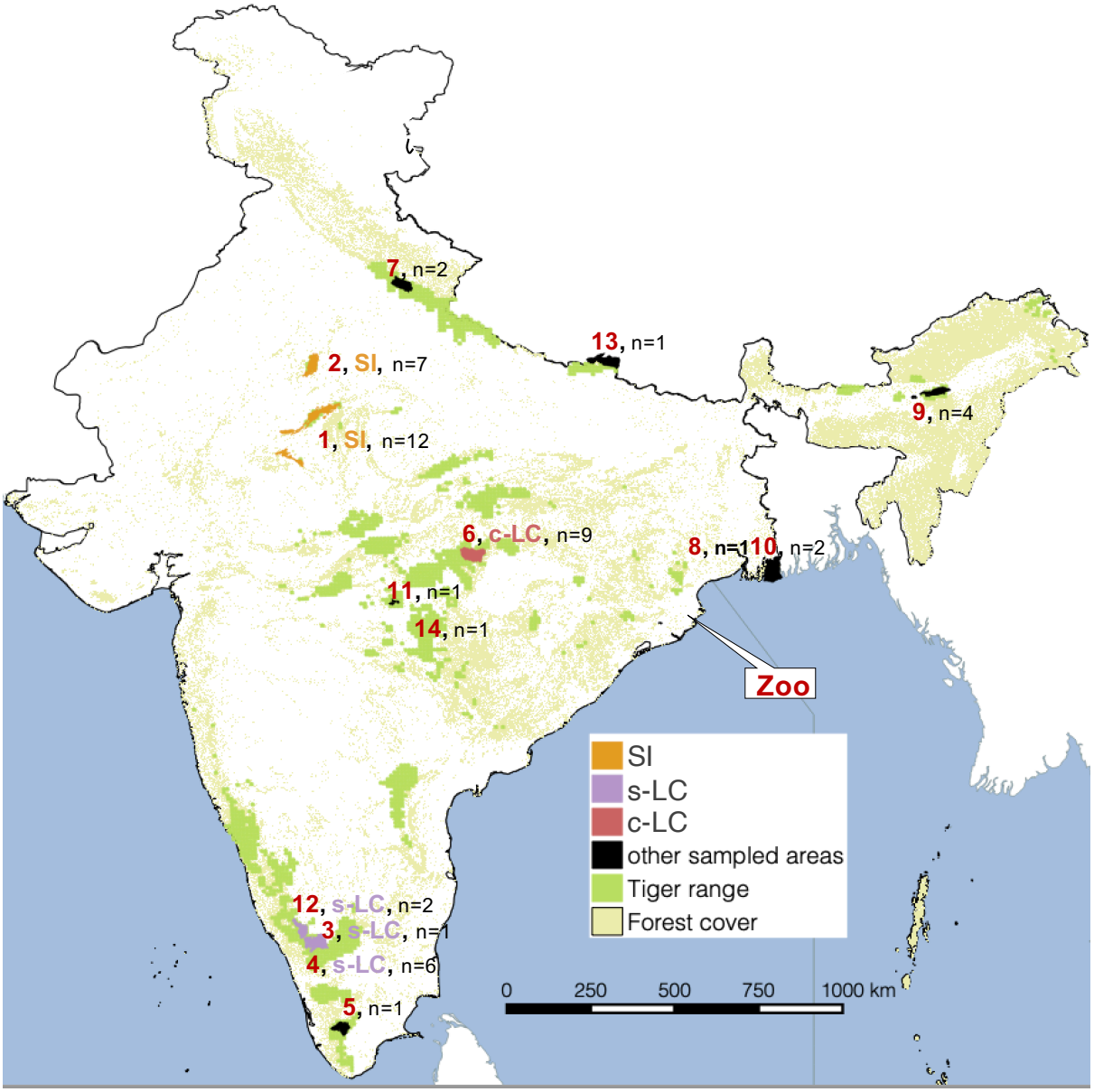

Figure 5. Sample locations and populations. The numbers in the figures represent the protected area where, North-west population: 1=Ranthambore Tiger Reserve (n=11), 2=Sariska Tiger Reserve (n=7), South India population: 3=Bandipur Tiger Reserve (n=3), 4=Wayanad Wildlife Sanctuary (n=6), 12=Nagarhole Tiger Reserve (n=2), 5=Periyar Tiger Reserve (n=1), Central Cluster: 6=Kanha Tiger Reserve (n=9), 7=Corbett Tiger Reserve (n=2), 8=Lalgarh Range (n=1), 10=Sunderban Tiger Reserve (n=2), 11=Bor Tiger Reserve (n=1), 13= Chitwan National Park (n=1), 14=Chandrapur (n=1), North East population: 9=Kaziranga Tiger Reserve (n=4) , Zoo: Zoo=Nanadankanan Zoo (n=5), Sagar et al. 2021.

528 *DNA extraction and sequencing*

529 We extracted DNA using Qiagen blood and tissue extraction kit (Cat. 69504) as per
530 the manufacturer's instructions. We prepared DNA whole genome sequencing
531 libraries using NEBNext UltraTM II DNA Library Prep Kit (Cat. E7645L, NEB Inc.).
532 We quantified DNA on QubitTM 3.0 fluorometer using Qubit High sensitivity dsDNA
533 Assay (#Q32854, Thermo Fisher Scientific). We then fragmented the quantified DNA
534 by sonication using Covaris LE220 ultrasonicator and Covaris microTUBE (#520053,
535 Covaris® Inc.) to obtain a final insert size of 250–350 bp. Next, we performed end-
536 repair on the DNA fragments where blunt ends are created on either side of the
537 fragments. We added a single "A" nucleotide on the 3′ ends of the fragments to
538 facilitate the ligation of NEB stem-loop adapters. We cleaned the ligated products
539 and size selected using Agencourt AMPure XP beads (#A63882, Beckman Coulter).
540 We amplified these size selected products using an eight-cycle PCR during which
541 indices (Barcodes) and flow-cell binding sequences were added. After a final clean-
542 up with Agencourt AMPure XP beads, we quantified the libraries using Qubit DNA
543 Assay and assessed the fragments using DNA TapeStation D1000 Screen Tape
544 (#5067-5582,5583, Agilent Technologies). We finally clonally amplified the quantified
545 libraries on a cBOT and sequenced them on the HiSeq X with 150bp paired end
546 chemistry.

547 *Whole Genome Re-sequencing and variant discovery*

548 We trimmed the raw reads using TRIMMOMATIC[87] to have an mean PHRED-scaled
549 quality of 30 in a sliding window of 15 bp, and removed any read that was shorter
550 than 36 bp after trimming from further analysis. We aligned these reads to a Bengal
551 tiger reference genome (JAHFZI000000000) using BOWTIE2[88]. The alignments
552 were then saved in a binary format (BAM) using SAMTOOLS1.9[89]. We marked
553 duplicate reads with the Picard Tools `MarkDuplicates` command
554 (http://broadinstitute.github.io/picard). We called variants from the BAM files using
555 Strelka with default options[90]. The variants were filtered with VCFtools[91] to retain
556 biallelic sites with a minimum minor allele count of 3, remove indels and loci with
557 mean depth across individuals below 2.5 percentile and above 97.5 percentile

558 across all loci. We removed all sites with missingness > 20% after removing
559 genotypes with genotype quality (GQ) less than 30. We removed the X chromosome
560 from the analysis. 1601148 SNPs with mean read depth = 17.2 (+/- 1.8) remained for
561 analyses after these filtering steps.

562 *Runs of Homozygosity*

563 We identified ROH using a sliding window, likelihood ratio method[92,93]. For this
564 analysis we used SNP data without the minimum genotype quality filter described
565 above, and then used genotype likelihoods, rather than called genotypes, as input in
566 order to maximize the genomic information available for detecting ROH. We split
567 each chromosome into sliding windows of 200 SNPs and step size of 10 SNPs. For
568 each individual *i* and window *j*, we calculated the probability of the observed
569 genotype likelihood at each SNP *k* (G_k) assuming the two alleles were IBD, and also
570 assuming they were non-IBD (Wang et al. 2009). We calculated the LOD score for
571 each window as

572 $$LOD(j,i) = \sum_{k=1}^{k_i} log_{10}\left(\frac{Pr(G_k|IBD)}{Pr(G_k|non-BD)}\right),$$

573 LOD scores across all windows and individuals form a bimodal distribution. Windows
574 with LOD score ≥ 0 were called IBD, while windows in the left hand side of the
575 distribution were called non-IBD as in Kardos et al.[44,94]. Consecutive IBD windows
576 were joined to map the starting and stop positions of ROH for each individual. F_{ROH}
577 was calculated as the fraction of the autosomal genome in ROH for example,

578 $$F_{ROH>100Kb} = \frac{\sum ROH_{>100Kb}}{Total\ autosomal\ size}$$

579 We calculated the percent of the genome in ROH above a particular size class to
580 estimate inbreeding arising from ancestors in different historical time periods. The
581 coalescent times of ROH were estimated as *g=100/(2rL)* where *g* is the expected
582 time (in generations) back to the parental common ancestor where the IBD

583 haplotypes forming an ROH coalesce[95], *r* is the recombination rate (here, 1.9cM per
584 Mb based on domestic cat[45]) and *L* is the length of the ROH in Mb. For example, a
585 100Kb ROH are estimated to arise from a single haplotype in an ancestor 263
586 generations ago on average. An individual's genomic inbreeding arising from
587 ancestors up to 263 generations ago is then estimated using only ROH longer than
588 100 kb. Similar calculations were performed for other ROH lengths. Table 1 lists the
589 lengths of ROH and corresponding average estimated coalescent times for the IBD
590 haplotypes that form the ROH.

591 Table 1. ROH lengths and the corresponding expected mean time of their origin due
592 to inbreeding where number of years assumes a generation length of 5 years.

ROH length(s)	generations since parents shared ancestor	years since parents shared ancestor
<100Kb	More than 263	More than 1315
>100Kb	Up to 263	Up to 1315
>1Mb	Up to 26	Up to 130
>5Mb	Up to 5	Up to 25
>10Mb	Up to 3	Up to 15

593

594 We constructed percentile bootstrap confidence intervals for mean F_{ROH} in each
595 population[96]. We randomly resampled individuals within a population with
596 replacement 10,000 times, each time calculating the mean F_{ROH} as described above.
597 The 0.025 and 0.975 quantiles defined the 95% confidence interval for mean F_{ROH} in
598 each population. We used a randomization procedure to test for a difference in the
599 mean F_{ROH} for each pair of populations as follows. First, we randomized the
600 population affiliation for each individual 10,000 times. For each randomization
601 replicate, we calculated the mean F_{ROH} for each of the two populations. We
602 calculated the *P*-value as the proportion of 10,000 randomization replicates where
603 the difference in F_{ROH} was at least as large as the empirical difference in F_{ROH}.

604 *IBD stretches of genome shared between pairs of individuals*

605 We estimated stretches of genome identical by descent (IBD) between pairs of
606 individuals using *IBDseq* default parameters[97]. We filtered for shared stretches
607 longer than 1Mb and estimated sum of shared lengths of more than 1Mb per
608 autosomal genome size. We estimated this for all pairs of individuals within a
609 population and all pairs across populations.

610 *Estimating effective population size from ROH*

611 We used the estimates of F_{ROH} using ROH arising from ancestors in 4 different
612 estimated time frames (Figure 1) to estimate N_e over each respective time frame. If
613 the estimated coalescent times for ROH are unbiased, then the average F_{ROH} based
614 on ROH with estimated maximum coalescent times less than t generations back in
615 time is an estimator of the inbreeding accumulated in the population from the time of
616 sampling back to t generations ago. We estimated N_e from the following expression
617 of mean expected individual inbreeding as a function of N_e over t generations

618 $$F_{ROH,t} = 1 - \left(1 - \frac{1}{2N_e}\right)^t.$$

619 This approach assumes that the probability of inferring an ROH segment due to
620 several smaller homozygous segments is very low. We calculated 95% confidence
621 intervals for N_e by bootstrapping over individuals within each population as described
622 above for our estimates of mean F_{ROH}.

623 *Mitochondrial haplotype diversity*

624 We used the reads aligned to the mitochondrial scaffold of BenTig1.0. We called
625 consensus fasta files for each individual from this as described in Khan et al.[81].
626 These multiple consensus fasta files were aligned using clustal omega
627 (https://www.ebi.ac.uk/Tools/msa/clustalo/) and written to nexus output files. We then
628 used DnaSP 6[98] to estimate haplotype diversity.

629 *Identifying effect of mutations*

630 We re-filtered the SNPs as described above with VCFtools[91], except here we
631 removed genotypes with phred-scaled genotype quality <30, and then removed loci
632 that were monomorphic, had >20% missing data, or read depth in the bottom and
633 top 2.5% quantiles for mean read depth across individuals. We also removed loci
634 and with extreme values of F_{IS} (F_{IS}<-0.5 or F_{IS} >0.95) estimated across all Indian
635 tigers to remove loci likely frequent genotyping or read mapping errors; this
636 expectedly resulted in mean F_{IS}>0 (0.14).

637 We used Ensembl Variant Effect Predictor[59] on SNPs to identify consequence of a
638 mutation and classify them as missense, loss-of-function (LOF) or intergenic as
639 described by Xue et al.[28]. We used annotation files generated for the Bengal tiger
640 assembly using RNA seq.

641 *Mutation load*

642 We used the R_{XY} method described by Do et al.[62] and implemented by Xue et al.[28] to
643 estimate relative excess of mutation loads in a population X with respect to another
644 population Y. We randomly selected 7 individuals from each population and
645 estimated the relative mutation load for each of the three possible pairwise
646 combinations of the S-I, s-L-C, c-L-C populations. Standard deviations were obtained
647 by 100 rounds of jackknife. For each round of jackknife for missense and LOF
648 mutations, we randomly excluded 10-15% of loci and repeated the estimation. For
649 intergenic variants, 100,000 loci were randomly excluded for each round.
650
651 To infer the ancestral state of each site in the tiger reference we converted the
652 references for the Domestic cat (GenBank: GCA_000181335.4), Cheetah
653 (GCA_003709585.1) and Lion (GCA_008795835.1) into FASTQ reads by sliding
654 across the genome in non-overlapping windows of 100 base pairs and transforming
655 each window into a separate FASTQ read. The resulting FASTQ reads were then
656 mapped to the tiger reference genome with bwa mem v0.7.17, slightly lowering the
657 mismatch penalty (*-B* 3) and removing reads that mapped to multiple regions.
658 Mapped reads were realigned around indels using GATK *IndelRealigner*. Next, we
659 converted the mapped reads into a haploid FASTA consensus sequence, excluding

660 all sites with depth above one (as such sites contain at least one mismapped read)
661 using ANGSD *-dofasta*. The ancestral allele at a locus was then determined as the
662 majority allele found in genomic alignment of the domestic cat, cheetah and lion.
663 Sites where the ancestral allele could not be identified (e.g., where the ancestral
664 identified in the cat-cheetah-lion alignment was not present in our sample of tigers)
665 were excluded.
666 We counted the number of homozygous deleterious alleles per individual as number
667 of derived missense and LOF mutations. We quantified uncertainty in the mean
668 number of homozygous deleterious alleles for each population using percentile
669 bootstrap confidence intervals as described above. We used randomization tests as
670 described above to test for differences in the mean number of homozygous
671 damaging and LOF alleles per individual for each pair of populations.

672 *Site-frequency spectrum*

673 We equalized the sample sizes across the populations, and across loci within each
674 population by randomly subsampling 16 non-missing alleles from each locus in each
675 population population before estimating the derived neutral and derived damaging
676 allele frequencies. For the SFS analysis of all populations combined, we subsampled
677 88 non-missing alleles from each locus. Repeating the subsampling process and
678 analysis had no effect on the results.

679 We used randomization tests to determine if the mean putatively damaging derived
680 allele frequency was statistically significantly different from the mean putatively
681 neutral derived allele frequency in each population, and for all populations combined
682 (Figure 3). We randomly reassigned the neutral versus damaging status for each
683 allele frequency 5,000 times. For each of the 5,000 replicates, we recalculated the
684 randomized mean derived neutral and damaging allele frequency. A *P*-value was
685 calculated as the proportion of the 5,000 permutations that resulted in a difference in
686 the mean neutral versus damaging derived allele frequency that was larger than
687 observed in the non-randomized data.

688 *Genetic rescue and F_{ST}*

We estimated genome wide genetic differentiation as F_{ST} with VCFtools. For loci with high frequency (>0.85, closer to fixation) deleterious (missense and LOF) alleles in a population, we listed all the loci in each target population (population in need of rescue). For every possible source population, we estimated the proportion of these loci with frequency < 0.85. We then calculated F_{ST} at only these loci to estimate damaging loci F_{ST}. The proportion or loci rescued was calculated as the fraction of loci that had < 0.85 frequency in the possible source population.

We investigate this for five possible source populations, including tigers from Kanha Tiger Reserve (KTR), Wayanad Wildlife Sanctuary (WAY), Ranthambore Tiger Reserve (RTR), Corbett Tiger Reserve (COR) and Kaziranga Tiger Reserve (KAZ).

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Data availability

All sequencing data have been deposited in BioProject PRJNA728665 in NCBI

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