1   **Small populations lose overall genomic diversity but can maintain adaptive potential**

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Abstract

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29   In principle, populations with higher genetic diversity and larger effective sizes have greater evolutionary capacity

30   (i.e., higher adaptive potential) to respond to ecological stressors. Small isolated populations tend to lose genetic

31   diversity rapidly due to the detrimental effects of drift and inbreeding via genetic erosion. We are interested in how

32   adaptive potential persists in small populations and how it fluctuates relative to overall genomic diversity. We

33   analyzed individual whole genome sequences from different populations of Montezuma Quail (*Cyrtonyx*

34   *montezumae*), a small ground-dwelling bird that is sustainably harvested in some portions of its range but is of

35   conservation concern elsewhere. Our results indicate that overall, Montezuma Quail populations in the U.S. exhibit

36   low levels of genomic diversity due in large part to long-term declines in effective population sizes over nearly a

37   million years. The smaller and more isolated Texas population is significantly more inbred than the large Arizona

38   and the intermediate-sized New Mexico populations. The Texas gene pool has a significantly lower proportion of

39   deleterious alleles than the Arizona gene pool, but also significantly more high-frequency deleterious alleles that,

40   coupled with elevated inbreeding, elevate the realized genetic load in Texas. Our results highlight that smaller,

41   isolated populations are at higher risk of inbreeding depression as detrimental mutations rise in frequency due to

42   drift and weakened purifying selection. Our study illustrates how population genomics can be used to proactively

43   assess both neutral and adaptive aspects of contemporary genetic diversity in a conservation framework while

44   simultaneously considering deeper demographic histories.

45

Introduction

46

47   Many species and populations world-wide are declining at an alarming rate (Barnosky et al., 2011;

48   Ceballos et al., 2015; Dirzo et al., 2014), mainly driven by human-mediated habitat loss and climate change (Loarie

49   et al., 2009). Active prevention of population declines and extirpations is a priority for conservation (Cardinale et

50   al., 2012; Thompson, Koshkina, Burgman, Butchart, & Stone, 2017) because reduction in population size is often

51   followed by reduction in genetic diversity (Allendorf, Luikart, & Aitken, 2013; Soulé, 1985). The loss of genetic

52   diversity has negative consequences on the survivability and future persistence of a species as it impedes their ability

53   to adapt to environmental change (Bijlsma & Loeschcke, 2005; Bürger & Lynch, 1995; Reed & Frankham, 2003).

54   Smaller and/or isolated populations exhibit a more rapid loss of within-population genetic variation as compared to

55   their larger counterparts (Willi, Van Buskirk, & Hoffmann, 2006). The combined effects of drift, inbreeding, weak

56   selection, and lack of gene flow in small, isolated populations may lead to "genetic erosion" (Bijlsma & Loeschcke,

57   2012). Genetic erosion is expected to indirectly reduce the mean fitness of a population and thus increase extinction

58   risks (Bijlsma & Loeschcke, 2012; Leroy et al., 2018). The impact of genetic erosion on the adaptive potential of a

59   species is increasingly recognized as an integral aspect of comprehensive conservation efforts (Holderegger et al.,

60   2019; Ralls et al., 2018).

61   Compared to large populations, small populations have less genetic diversity and are more prone to risks of

62   inbreeding depression as they are burdened with higher proportions of detrimental mutations ("genetic load") in

63   their protein-coding genes (Lynch, Conery, & Burger, 1995). Genetic erosion in small populations can lead to an

64   increase in the frequencies of partially recessive deleterious alleles (Charlesworth, Morgan, & Charlesworth, 1993;

65   Lynch, 2007) which can lead to inbreeding depression in inbred populations (Keller, 2002). In theory, larger

66   populations are more efficient at purging the genetic load of deleterious alleles via stronger purifying selection

67   (Hedrick & Garcia-Dorado, 2016), but the empirical evidence for such purging is mostly experimental (Bersabé &

68   García-Dorado, 2013; Bijlsma, Bundgaard, & Van, 1999; Crnokrak & Barrett, 2002) and rarely explored in natural

69   populations (Rettelbach, Nater, & Ellegren, 2019).

70   Much of the vertebrate genome has no known benefit to its host and is thought to evolve in a nearly-neutral

71   fashion (Ohta, 1992). Much of the genome (e.g., intergenic regions) can be shaped by genome-wide processes such

72   as inbreeding, migration, and demographic stochasticity (Pool & Nielsen, 2007). In contrast, genetic variants that

73   exist in genic regions may be important for adaptation and thus subject to targeted, locus-specific natural selection.

74   The type and strength of selection on such adaptive variants largely determines the phenotypic response (Ellegren &

75   Sheldon, 2008). Contemporary genomic patterns of diversity are shaped by both recent and historic factors such as

76   lack of gene flow due to anthropogenic habitat fragmentation (Lino, Fonseca, Rojas, Fischer, & Ramos Pereira,

77   2019) and demographic responses to glaciations (Nadachowska-Brzyska, Li, Smeds, Zhang, & Ellegren, 2015),

78   respectively. Hence, explicitly comparing whole genomes with defined genic regions should help with identifying

79   the major contributors to overall genomic architecture and also evaluate the adaptive potential of populations. In this

80   study, we use whole genome sequences to quantify genic and whole genome variation from different sized

81 populations of Montezuma Quail (*Cyrtonyx montezumae*), then estimate the degree of genetic erosion and its impact

82 on adaptive potential.

83 The Montezuma Quail is a small gamebird that is hunted in portions of Mexico, New Mexico, and Arizona

84 but of conservation concern in Texas (Fig. 1). It is one of the least-studied avian species in North America

85 (Gonzalez, Harveson, & Luna, 2015) due to its cryptic nature as well as difficulties associated with live trapping and

86 monitoring (Hernandez, Harveson, & Brewer, 2006). Montezuma Quail are currently experiencing species-wide

87 declines within the U.S. (Harveson et al., 2007), and Texas populations are listed as Vulnerable by Texas Parks and

88 Wildlife Department (TPWD) with no open hunting season due to growing concerns about extirpations (Harveson,

89 2009). Unlike other North American quails, Montezuma Quail are diet (Albers & Gehlbach, 1990) and habitat

90 specialists (Brown, 1979) that heavily rely on grass cover for predator evasion (Bristow & Ockenfels, 2004). Their

91 demography is strongly impacted by seasonal rainfall (Chavarria, Montoya, Silvy, & Lopez, 2012) and adequate

92 grass cover (Brown, 1979) making habitat degradation and fragmentation major threats to Montezuma quail survival

93 (Luna, Oaster, Cork, & O'Shaughnessy, 2017). Populations in Arizona are more genetically diverse than those from

94 Texas or New Mexico (Mathur, Tomeček, Heniff, Luna, & DeWoody, 2019) and are expected to be the least

95 impacted by genetic erosion due to larger sizes and more contiguous habitat (Fig. 1). In contrast, the Texas

96 population is expected to have the highest signature of genetic erosion due to a restricted geographic range and

97 associated demographic isolation.

98 Herein, we report the data from whole genomes sequencing (WGS) of 90 Montezuma Quail from Arizona,

99 New Mexico, and Texas. We used these WGS data to quantify the levels of overall genomic diversity, genic (i.e.,

100 adaptive) variation, differentiation, individual inbreeding, and the genetic load in each population. We do so in a

101 conservation context by comparing populations of different sizes. Our results indicate that Montezuma Quail

102 effective population sizes have decreased over much of the last million years, and their similar trajectories over time

103 indicate that now-disjunct populations in the U.S. were long connected demographically. Furthermore, we find that

104 the small Texas population is isolated, inbred, genetically depauperate, and more prone to inbreeding depression.

105

106 **Materials and Methods**

107 *Samples, DNA extraction, and sequencing*

108   Montezuma Quail samples were opportunistically collected from three representative geographic

109   populations in the United States: Arizona (AZ), New Mexico (NM), and Texas (TX) as described earlier (Mathur et

110   al. 2019; Fig. 1). Based on the size of their geographic range in each state, on assessments by each state game

111   agency, on eBird sightings, and on previous genetic analyses, we explicitly assume that AZ samples come from a

112   large population, NM from a medium-sized population, and TX from a small population relative to each other

113   (Mathur et al. 2019). Arizona samples were acquired from hunter harvested wings initially collected by Randel et al.

114   (2019). New Mexico samples were acquired as voucher specimens by R. Luna, whereas Texas samples were

115   collected as road-kill carcasses by L. Harveson. Sample handling and DNA extraction protocols are described in

116   Mathur et al. (2019).

117   We sequenced whole genomes of 90 Montezuma quail samples (AZ=60, TX=17, NM=13) by creating

118   individually barcoded dual-index libraries using Illumina® Nextera™ reagents following the manufacturer's

119   protocol. The libraries were sequenced in 8 lanes of paired-end 150bp reads (2x150bp) on one S4 flow cell using

120   Illumina® NovaSeq™ 6000 sequencing system in Purdue University's Genomics Core Facility. We removed any

121   sample if they failed to generate more than 8 million reads (i.e. less than 1x mean read depth).

122

123   *Sequencing filtering, alignment, and read preprocessing*

124   We used FastQC v0.11.7 (Andrews, 2010) to quality check our raw reads and removed adapter sequences

125   from trailing and leading edges of each read using Trimmomatic v.036 (Bolger, Lohse, & Usadel, 2014). We also

126   used Trimmomatic to remove low quality sequences (Phred < 20) and any read smaller than 30bp after clipping and

127   quality filtering, prior to any further downstream analysis.

128   The filtered reads were mapped to a Montezuma quail draft genome assembly (Mathur et al. 2019) with

129   BWA v.0.7.17 (Li & Durbin, 2009) using the *mem* algorithm. Samples with less than 50% mapped reads were

130   removed from further analysis. Our final dataset contained 74 individuals (AZ=52, TX=15, NM=7). We used the

131   Genome Analysis ToolKit (GATK) "Best Practice Workflow" (Auwera et al., 2018) to pre-process our mapped

132   reads. We first sorted the reads by their co-ordinates and marked duplicates using PicardTools

133   (http://picard.sourceforge.net). We then used GATK v3.6.0 (McKenna et al., 2010) to realign our reads around

134   indels to minimize misaligning with mismatches. We identified the regions to be realigned using

135   RealignerTargetCreator and aligned bam files using IndelRealigner. The base quality score was recalibrated for all

136   the reads using known variant sites discovered from high coverage genome reads (Mathur et al., 2019) using

137   BaseRecalibrator. We finally used these filtered-realigned-recalibrated reads to get coverage statistics using

138   samtools depth (Li et al., 2009), and for further downstream analyses.

139   In cases where we needed to polarize genomic variants as ancestral or derived (see below), we used the

140   high-quality and contiguous chicken genome (*Gallus gallus* GRCg6a) as reference. Both Galliformes, Montezuma

141   Quail belong to the New World quail Family Odontophoridae that diverged from junglefowl (*Gallus* spp.; Family:

142   Phasianidae) approximately 30-40 million years ago (Cox, Kimball, Braun, & Klicka, 2007; Hosner, Braun, &

143   Kimball, 2015). Read mapping and preprocessing steps were same as above.

144

145   *Mitogenome assembly and diversity*

146   We mapped genomic reads to the previously published Montezuma Quail mitogenome (Mathur et al.,

147   2019) and extracted the uniquely mapped reads (mito-reads) using BBMap v37.93 (Bushnell, 2014). Since nuclear

148   copies of mitochondrial DNA (NUMTs) exist in nearly all eukaryotic genomes (Bensasson, Zhang, Hartl, & Hewitt,

149   2001; Lopez, Yuhki, Masuda, Modi, & O'Brien, 1994), we tried to first identify the NUMTs in the nuclear genome

150   assembly of the Montezuma Quail. We used a BLAST-based approach to query the Montezuma Quail reference

151   mitogenome against a custom blast database of Montezuma Quail nuclear genome scaffolds. We extracted the

152   NUMT sequences from genome assembly as fasta files using faSomeRecords (Kent et al., 2002). Any mito-read that

153   also uniquely matched to the NUMT fasta sequences were removed using BBMap. This helped ensure that final

154   mito-specific reads we retained belonged to the mitogenome and not NUMTs. We used samtools mpileup to align

155   mito-specific reads to the reference mitogenome and used bcftools (Li et al., 2009) to call variants. We filtered the

156   variants with a minimum base depth of 10 using vcflib (Garrison, 2012) and used bcftools consensus to create

157   consensus mitogenomes for every individual. To avoid mismapping and errors introduced at the artificial ends

158   created in the linearized mitogenome, we trimmed 40bp from either end of the mitochondrial sequence prior to

159   analysis.

160   All mitogenomes were aligned as multiple sequence alignment using Clustalw v.2.1 (Thompson, Higgins,

161   & Gibson, 1994) using default parameters. We calculated mitochondrial nucleotide diversity indices and haplotype

162   statistics using Arlequin v3.5 (Excoffier & Lischer, 2010). We accounted for unequal sample sizes for each

163   population by randomly subsampling mitochondrial genomes from each population (N=7) and recalculated

164   nucleotide diversity indices using 100 independent permutations.

165

166   *Genotype likelihood estimation, subsampling, and genotype calling*

167   For the nuclear reads, we used ANGSD v0.929 (Korneliussen, Albrechtsen, & Nielsen, 2014) to estimate

168   genotype likelihoods (GL) call single nucleotide polymorphisms (SNPs) using the samtools model. We filtered bam

169   files to only include unique reads with a minimum mapping quality of 50. We excluded bases with a base quality

170   score < 20 and only retained only proper pairs. Major and minor allele was inferred from the GL and triallelic sites

171   were removed. Per-site allele frequencies (AF) were estimated using a combination of estimators i.e. first estimating

172   allele frequency from GL assuming both major and minor alleles are known and then re-estimating AF by summing

173   over the three possible minor alleles weighted by their probabilities. We used a p-value cut off of 10^{-6} to call a site

174   polymorphic and a minimum minor allele frequency (MAF) of 0.05. We also used a maximum depth threshold of

175   500 to avoid calling SNPs from repetitive regions (Clucas, Lou, Therkildsen, & Kovach, 2019). Deviations from

176   Hardy-Weinberg equilibrium were tested and sites with p-value < 0.01 were filtered out to remove potential

177   paralogous sequences with an excess of heterozygotes due to erroneous mapping (Meisner & Albrechtsen, 2019).

178   When estimating GL across all samples (N=74), we used a threshold of minimum 60 individuals to ensure

179   including segregating sites from more than one population, in other words, to prevent retaining sites from only the

180   Arizona population (N=52) ("population dataset"). To avoid biases introduced due to uneven sample sizes, we re-

181   estimated GL and discovered SNPs from an equal subset of Arizona and Texas samples (N=21; AZ=7, TX=7,

182   NM=7). For our subsamples, we chose samples with the highest depth and breadth of coverage to maximize the

183   genomic spread of our variants ("genomic dataset"). For the subset, we used a minimum individual threshold of 15

184   and maximum depth threshold of 100.

185   In the end, we analyzed our genotype likelihood data two ways: (a) retaining maximum individual

186   information at the cost of markers per individual ("population dataset") (McLennan, Wright, Belov, Hogg, &

187   Grueber, 2019) and (b) retaining maximum genomic information of each population at the cost of individuals

188   analyzed per population ("genomic dataset") (Benjelloun et al., 2019). The population dataset was used for the

189   estimation of inbreeding and genetic structure, both of which can be inferred from a smaller set of widespread

190   markers from more individuals, whereas the genomic dataset with higher SNP density was used to estimate genome-

191   wide diversity and for detecting signatures of selection.

192

193   *Relatedness, inbreeding co-efficient, and population structure estimation*

194   Assumptions of many population genetic estimators are violated if family members and closely related

195   individuals are analyzed simultaneously. Related individuals among a sample set should thus be identified and

196   removed prior to population structure analysis (Meisner & Albrechtsen, 2018, 2019). We estimated relatedness

197   among our samples using IBSrelate (Waples, Albrechtsen, & Moltke, 2019). IBSrelate uses GL estimates to

198   categorize a pair of individuals as either parent-offspring, full-siblings, half-siblings, first-cousins, or unrelated

199   based on whether the pair share the same genotype or exhibit dissimilar genotypes at a particular site (Manichaikul

200   et al., 2010). We compared all individual pairs (total of 2701 comparisons) and removed any pairwise comparison

201   from relatedness estimates if the number of sites compared were less than 100,000.

202   We estimated individual inbreeding coefficient (F) using PCAngsd v.0.982 (Meisner & Albrechtsen, 2018)

203   from inferred GL. This allows F-values at a site to vary between -1 and 1 where a negative value indicates an excess

204   of heterozygotes and a positive value indicates an excess of homozygotes at a site. Since inbred individuals would

205   have an excess of homozygous sites, they should have an overall $F > 0$. We used extremely low tolerance values

206   (1e-9) and 5000 maximum iterations for estimation to assure a stricter stopping criterion and avoid convergence at a

207   local minimum (Fig. S10).

208   To identify genetic structure in our Montezuma Quail samples, we used two approaches. First, we used

209   PCAngsd to calculate a covariance matrix and performed individual level PCA using princomp function in R (Team,

210   2013). Second, we used NGSAdmix (Skotte, Korneliussen, & Albrechtsen, 2013) to estimate individual admixture

211   proportions. For PCAngsd, we used a minimum tolerance value for population AF estimation of 1e-9, a tolerance

212   threshold for updating individual AF of 1e-9 for 1000 iterations. For NGSAdmix, we ran 10 independent runs for

213   each K from 1-10 with minimum MAF 0.05, 1e-9 tolerance for convergence, 1e-9 tolerance for log likelihood

214   difference in 50 iterations, and maximum 50,000 iterations. The most likely number of subpopulations were

215 determined based on first and second order rate of change of the likelihood distribution from the 10 runs (Evanno,

216 Regnaut, & Goudet, 2005).

217

218 *Nucleotide diversity, heterozygosity, and contemporary effective population size estimation*

219 For nucleotide diversity estimates, we only used the genomic dataset to avoid biases in estimating site

220 frequency spectrum (SFS) due to uneven sample sizes and heavy data pruning, which was the case for our

221 population dataset. We used ANGSD to generate a folded SFS by using the Montezuma Quail reference genome and

222 a minimum base quality of 20 and minimum mapping quality of 50. Next, we obtained a maximum likelihood

223 estimate of the SFS using realSFS by bootstrapping it 1000 times and using the mean SFS for each population to

224 estimate per-site Watterson's theta (θ_W). We obtained mean heterozygosity (H_o) and its variance from the

225 bootstrapped SFS.

226 To obtain an estimate of contemporary effective population sizes (N_e) from mean genomic θ_W, we first

227 estimated the whole-genomic mutation rate (μ) for Montezuma Quail ($\theta_W = 4N_e\mu$). Since no linkage map exists for

228 Montezuma Quail, we estimated μ following Zhan et al. (2013). The Montezuma Quail reference assembly was

229 mapped to the Chicken genome (*Gallus gallus* GRCg6a) using LASTZ (Harris, 2007). The mean divergence time (t)

230 between chicken and Montezuma Quail was derived from www.timetree.org and polymorphic loci were identified

231 only if neither target nor query nucleotide was N/n and the locus was not in an alignment gap. The final μ per nt per

232 year was calculated with the following formula: μ = (counts of mutated loci / sequence length) / 2t (Zhan et al.,

233 2013).

234

235 *Genetic differentiation and signatures of local adaptation*

236 Small populations in isolation drift apart randomly at some loci (Whitlock, Ingvarsson, & Hatfield, 2000)

237 and can become locally adapted to their microenvironment at adaptive loci (Schierup et al., 2018). Both processes of

238 drift and adaptation lead to nucleotide divergence (D_{XY}) and variation in allele frequencies (F_{ST}) among populations

239 (Matthey-Doret & Whitlock, 2019; Puzey, Willis, & Kelly, 2017; Rousset, 1997). We investigated genomic patterns

240 of genetic differentiation by estimating pairwise F_{ST} using a sliding window approach (window size=100kb,

241 step=50kb) for each population pair (AZ-TX, TX-NM, AZ-NM). We used ANGSD to calculate the 2D SFS for each

242   population pair using the chicken genome (GRCg6a) as reference to polarize alleles as derived or ancestral. We

243   quantified the levels of nucleotide divergence (D_{XY}) using the calcDxy.R

244   (https://github.com/mfumagalli/ngsPopGen/blob/master/scripts/calcDxy.R). In this case, we estimated GL for each

245   population individually, but only retained sites that were shown to be segregating in all populations. This ensured

246   that sites with a fixed allele in one population is still included in our per population D_{XY} calculations.

247   To identify candidate regions under putative selection due to local adaptation, we Z-transformed F_{ST} around

248   the mean for each sliding window and examined the outliers that had $Z(F_{ST})$ values outside 5 standard deviations

249   from the mean (Willoughby, Harder, Tennessen, Scribner, & Christie, 2018). After removing false positives that

250   showed higher deviations due to lack of data, the remaining outlier windows were inspected for nearby genes. We

251   blasted the 100-kb outlier window to the chicken genome using default parameters and only retained windows that

252   contained annotated genes with known function.

253

254   *Adaptive diversity, and adaptive differentiation estimation*

255   The Montezuma Quail genome consists of ~17,500 genes (Mathur et al., 2019) and we examined levels of

256   variation in just the genic regions as a comparison to the genome-wide estimates of variation. We filtered out reads

257   that mapped to the Montezuma Quail gene sequences using BBMap. The genotype likelihoods and diversity indices

258   were estimated for the genic regions using genic reads following the same methods and parameters as above.

259   To investigate levels of differentiation within the genes, we used population branch statistics (PBS) in

260   ANGSD. PBS are based on comparing the pairwise F_{ST} values between the three sampled populations and identify

261   the directionality of sequence differentiation (Yi et al. 2010) along each branch of their corresponding three-

262   population tree. PBS represents the amount of allele frequency change at a locus in a population's history since its

263   divergence from the other two populations and have the power to detect recent selection (G. Amorim et al., 2017;

264   Jiang & Assis, 2019). We computed mean PBS for each annotated gene (N=17,573) in the Montezuma Quail

265   genome to determine if Texas quail have genes that are exceptionally diverged relative to the other two populations,

266   possibly due to positive selection (Yi et al., 2010).

267

268   *Population trends and historic demographic sizes*

269   A population that underwent a bottleneck should lose rare variants and leave intermediate frequency

270   variants in the genome, resulting in an overall positive value of Fu's F statistic (Fu, 1997). On the other hand, new

271   mutations would be added in an expanding population resulting in excess of rare variants and a negative mean value

272   of Fu's F. Fu's F is more sensitive to demographic changes than Tajima's D (Ramos-Onsins & Rozas, 2002) but

273   requires ancestral sequences for unbiased estimations. Thus, we estimated mean Fu's F statistic for every population

274   over a sliding window in ANGSD using the chicken genome as an ancestral reference with 100kb window size and

275   50kb step.

276   We reconstructed ancestral demographic histories using SMC++ v.1.15.2 (Terhorst, Kamm, & Song, 2016)

277   which uses unphased whole genome data to infer population size histories using sequential Markov coalescent

278   (SMCs) simulations. The reads that mapped to the first 10 chicken chromosomes (NC_006088.5- NC_006097.5)

279   comprising ~ 750 Mbp were used to create composite likelihoods for each population individually by varying the

280   identity of the distinguished individual while keep other individuals within the population as undistinguished. We

281   used cross-validation to estimate population size changes using the Powell algorithm with a tolerance of 1×10^{-5} and

282   a mutation rate of 3.14×10^{-09} (estimated as above). We ran our model using 5000 iterations and used different

283   parameter values for thinning and regularization penalty to avoid degeneracy in the likelihood and overfitting

284   (Terhorst et al., 2016) with final model generated using thinning parameter of 1300 and regularization penalty of 6.

285   A generation time of 1.5 was used to convert generations into years.

286

287   *Estimation of genetic load*

288   To assess the impact of a mutation in genic regions and its zygosity, we called genotypes at SNPs within

289   the genes from GL estimates. Genotypes were only called at sites with minimum individual depth of 5X to minimize

290   technical biases (Benjelloun et al., 2019). Allele frequencies from the genotype calls at each of the genic variants

291   were calculated using vcftools v.0.1.16 (Danecek et al., 2011). We predicted the effect of a variant on amino acid

292   change and its deleterious impact using SnpEff 4.2 (Cingolani et al., 2014). A variant is classified as either high,

293   moderate, low, or modifier based on its inferred effect on protein translation. We quantified the genetic load of a

294   population as the proportion of deleterious variants within the genic regions and compared the among-population

295   differences in such proportions using chi-squared tests with Yates' continuity correction (Newcombe, 1998; Yates,

296   1934). High impact variants have the most disruptive (i.e., deleterious) effect on protein like truncation or loss of

297   function, whereas low impact mutations are mostly synonymous with little to no impact on proteins (Cingolani et

298   al., 2014). Thus, high impact variants have the potential to impart the greatest genetic load (Ellegren & Sheldon,

299   2008) as compared to low or synonymous variants (Crnokrak & Barrett, 2002), but the realized genetic load depends

300   upon dominance and zygosity. As an extreme example, one can imagine a genome where 50% of the protein-coding

301   genes are compromised by high-impact variants (i.e., high potential load), but the realized genetic load is nil if they

302   are all recessive and outbreeding prevails. In contrast, a few high-impact deleterious recessive mutations (low

303   potential load) contribute greatly to the realized genetic load if they are homozygous due to inbreeding.

304

305   **Results**

306   In this study, we collected WGS data from 90 Montezuma Quail (AZ=60, TX=17, NM=13; Fig. 1). We

307   generated more than 1.65 billion reads (mean = 18.5 million reads per individual) corresponding to approximately

308   250 billion bases (mean = 2.8 billion bases per individual; >2x individual coverage). Since these samples were

309   opportunistically collected (i.e. either hunter-harvested wing tissues or roadkill carcasses), we found significant

310   variability in the quality and quantity of DNA sequenced. This stochasticity was evident from sequences generated

311   per individual (Table S1) and their depth and breadth of coverage (Table S1, Fig. S1). We removed samples that

312   failed to generate the threshold of 8 million bases (N=10) or where less than 50% of the total reads mapped to the

313   Montezuma Quail assembly (N=6). However, we achieved a high level of read mapping for the remainder of the

314   samples (84.4% ± 18.1%; Table S1). Ultimately, we analyzed genomic information from 74 individuals (AZ=52,

315   TX=15, NM=7) that covered 65.1 ± 22.1% (mean ± SD) of the Montezuma Quail genome at 2.1 ± 1.3X depth

316   (Table 1).

317   Our complete mitogenome analysis detected 39 unique haplotypes in the Arizona population with 239

318   parsimony-informative sites shared among them. There were 11 unique Texas haplotypes sharing 171 parsimony-

319   informative sites, and we found only 3 unique haplotypes for the New Mexico population with 167 such sites. We

320   found per-site nucleotide diversity (Π) and Kimura 2-P pairwise distances to be smaller in the Texas and New

321   Mexico mitogenomes (p=0.03 and p=0.04 respectively) as compared to Arizona. Haplotype diversity (H_d) did not

322 significantly differ between Texas and Arizona mitogenomes (p = 0.70) but was significantly smaller in New

323 Mexico as compared to Arizona (p=0.02; Fig. S2).

324   For the nuclear genome analysis, we partitioned our data into two datasets: population and genomic. The

325 population dataset consisted of genotype likelihoods from 456,373 SNPs retained from all individuals (N=74). The

326 genomic dataset contained genotype likelihood information from 6,696,145 SNPs sampled across an equal subset of

327 each representative population (N=21). Using the population dataset, we first estimated the relatedness among our

328 samples to determine if we had close relatives in the study. Pairwise relatedness was measured for 2,341 individual

329 pairs. Almost all the pairs analyzed were either unrelated (99.5%) or 3rd-degree relatives (0.21%). We found no full-

330 sibling or parent-offspring relationships (1st-degree) in our samples; however, 5 pairs from Arizona, 1 pair from

331 Texas, and 1 pair from New Mexico had 2nd degree or half-sibling relationship (Fig. 2A). Overall, our kinship

332 analysis indicates that, consistent with our opportunistic field sampling and broad survey range, close relatives were

333 only rarely sampled and thus, should not impact our population structure results. Inbreeding co-efficient estimates

334 (Table 1) showed significantly higher levels of mean inbreeding in Texas birds as compared to Arizona birds (Fig.

335 2B; Table S2) whereas inbreeding in Texas was only slightly elevated relative to New Mexico birds. Both PCA and

336 admixture analyses produced similar results indicating that the Arizona, Texas, New Mexico populations are

337 genetically distinct (Fig. 2C, D). However, based on the ΔK method (Evanno et al., 2005), the most likely number of

338 ancestral populations is K=4 (Fig. S3), splitting Arizona populations into two subpopulations (Fig. 2C). The

339 population-level trends for relatedness, inbreeding and genetic differentiation were concordant between the two

340 datasets (Fig. S4) and thus it seems clear that sampling issues have not biased our interpretations.

341   We used genomic dataset to quantify the levels of genome-wide nucleotide diversity as estimated by per-

342 site Watterson's theta (θ_W). Mean genome-wide θ_W was significantly lower for the Texas population (θ_W = 4.05 x

343 10^{-4}; SE = 1.67 x 10^{-7}) as compared to both Arizona (θ_W = 5.37 x 10^{-4}; SE = 1.93 x 10^{-7}) and New Mexico (θ_W = 4.57

344 x 10^{-4}; SE = 1.80 x 10^{-7}) (Table 1; Table S3). The genome-wide distribution of per scaffold diversity had higher a

345 mean in the Arizona population than in Texas or New Mexico (Fig. S5). Contemporary estimates of N_e were

346 quantified using whole-genomic μ of 3.14 x 10^{-9} bp^{-1}year^{-1} (CI: 2.59 x 10^{-9} - 3.34 x 10^{-9}) (Table 1). Thus, Texas quail

347 show a ~30% reduction in their overall genomic diversity with a mean, long-term evolutionary N_e reduction of

348 ~25% relative to Arizona. The genomic heterozygosity was also significantly reduced for Texas samples (Table 1)

349 as compared to either Arizona or New Mexico (Fig. 3A; Table S4). This indicates that smaller Montezuma Quail

350 populations in Texas and New Mexico are more severely impacted by genetic erosion with contemporary diversity

351 equivalent to those reported in endangered and vulnerable avian species, whereas the larger Arizona population has

352 heterozygosity estimates similar to other birds of least concern (Fig. 3B).

353   Global estimates of F_{ST} between each population pair showed low to moderate levels of genetic

354 differentiation at the whole genome level (Table 2). However, we found significant variation in F_{ST} values across the

355 genome for each population pair (Fig. 4; Fig. S6). One interesting observation was large $Z(F_{ST})$ scores for loci on

356 chromosome 16 (NC_006103.5) for all population comparisons (Fig. 4; Fig. S6). This is probably due to low

357 synteny between quail and chicken at chromosome 16 (Morris et al., 2020), perhaps due to an inversion (Clucas et

358 al., 2019) but this needs further validation using longer sequence scaffolds (Lamichhaney & Andersson, 2019).

359 There is a similar discontinuity at one end of chicken chromosome 26 (Fig. S6). We examined the windows that

360 were highly differentiated in both AZ-TX and TX-NM comparisons to look for genes and assess their functionality.

361 Genes or a gene clusters associated with the outlier peaks are shown in Fig. 4 and their known functions are listed in

362 Table S5. Per-site F_{ST} and D_{XY} values for SNPs located in those genes are in shown in Fig S7. In total, we found 12

363 genes that exhibited very high levels of differentiation (> 5 SD) and because these genes are associated with fitness-

364 related traits (Table S5), they are likely candidates for genes underlying local adaptation in Texas quail.

365   One of the major emphasis of our study was to assess the adaptive potential of Montezuma Quail,

366 particularly in the small, isolated Texas population. Variation in protein coding genes has the capacity to gauge

367 adaptive potential (Barbosa et al., 2018). The trends we observed for genic diversity were similar to the whole

368 genome (Table 1) with a 1.4X reduction of genic diversity in Texas quail. The Texas population had significantly

369 lower (θ_W = 3.49 x 10^{-4}; SE = 1.06 x 10^{-6}) genic nucleotide diversity as compared to Arizona (θ_W = 4.89 x 10^{-4}; SE =

370 1.25 x 10^{-6}) and New Mexico (θ_W = 3.68 x 10^{-4}; SE = 1.09 x 10^{-6}; Table S6). Mean heterozygosity in the genic

371 regions of Texas and New Mexico quail were both significantly reduced relative to Arizona quail whereas Texas and

372 New Mexico samples showed similar levels of genic heterozygosity (Fig. 3A; Table S7). One interesting

373 observation was that all three populations of Montezuma quail maintain more genic heterozygosity than genome-

374 wide heterozygosity (Fig. 3A, Table 1).

375   We inferred levels of adaptive differentiation using both pairwise F_{ST} and population branch statistics

376   (PBS). Consistent with our estimates of heterozygosity (Fig. 3), our F_{ST} estimates from the genic regions show

377   significantly higher levels of differentiation among the three populations as compared to the whole genomic

378   background (Table 2). Texas quail have a higher mean genic PBS than the other populations (Fig. S8; Table S8),

379   which may be indicative of local adaptation.

380   Demographic analysis indicated that the Arizona population is expanding with Fu's F = -0.23 ± 0.01 (mean

381   ± SE) whereas both the Texas and New Mexico populations are declining with Fu's F = 0.11 ± 0.02 and 0.22 ± 0.02

382   respectively (Fig. 5A). We tracked N_e estimates over the last ~1 million years using the pairwise sequentially

383   Markov coalescent method (Fig. 5B). The three populations display concordant trajectories for most of their

384   evolutionary history over that timeframe. We observed a decline in N_e from in the period of 10^6 - 10^5 years before

385   present (YBP) followed by a more stable period. A subsequent re-expansion occurred around 10,000 years ago, then

386   populations began to rebound until growth rates became negative around 3000-5000 YBP (Fig. 5B).

387   To quantify the potential genetic load of adaptive variants, we compared the mutations within protein-

388   coding genes (Fig. 6A) and their predicted change on translation (Fig. 6B). Most of the genic variation was due to

389   non-coding intronic variants or variants immediately outside the transcription unit (Fig. 6A), neither of which

390   influence amino acid sequences but both of which may impact expression levels. The Arizona population had a

391   significantly higher proportions of high, moderate, and low impact deleterious mutations when compared to either

392   the Texas or New Mexico populations (Fig. 6B; Table S9). These results show that the larger Arizona population

393   carries the greatest potential genetic load of deleterious mutations (Fig. 6B). However, the smaller and more inbred

394   Texas population exhibited those detrimental mutations at higher frequencies (Fig. 6C) and due to the elevated

395   inbreeding in Texas (Fig. 2B; Fig. S9) has the highest realized genetic load if we assume many of these deleterious

396   alleles adhere to a model of simple dominance. Overall, these data indicate that larger populations harbor

397   detrimental mutations at lower frequencies due to purging via stronger purifying selection and/or weaker purifying

398   selection in smaller populations (Hedrick & Garcia-Dorado, 2016; Keller, 2002). We acknowledge that frequency

399   estimates based on called genotypes may be biased due to low coverage and sample size (Benjelloun et al., 2019),

400   but the trends we observe here are exactly what is predicted by simulations (Coop et al., 2015) and seen in human

401   population studies (Do et al., 2015; Simons, Turchin, Pritchard, & Sella, 2014).

402

<u>Discussion</u>

In this study, we analyzed whole genome sequences from three natural populations of Montezuma Quail that vary in size and habitat continuity (Fig. 1) to understand how drivers of genetic erosion (e.g., small sizes and isolation) can affect genomic diversity and reservoirs of future adaptive potential. Small populations are predicted to have lower levels of diversity (Soulé, 1985) and recessive deleterious alleles should have a more pronounced impact on fitness than in large populations due to inbreeding (Charlesworth & Charlesworth, 1999). Populations that have experienced declines and are restricted to smaller habitats tend to have lower levels of overall genomic heterozygosity (Barsh, Rogers, & Slatkin, 2017; Brüniche-Olsen, Kellner, & DeWoody, 2019; Palkopoulou et al., 2015), but how these factors affect the adaptive potential is far less explored. By comparing levels of genome-wide diversity, adaptive (genic) diversity, and quantifying genetic load in different populations, our aim was to get a better understanding of how the adaptive potential of a species is affected by genetic erosion. The implications from our results should be relevant to conservation of Montezuma Quail and other small natural populations that are facing extinction threats worldwide (Ceballos et al., 2015).

Genetic erosion reduces genomic diversity

Our genomic diversity estimates are consistent with predictions for small declining populations that are expected to be most impacted by genetic erosion (Bijlsma & Loeschcke, 2012; Leroy et al., 2018). Species with small populations sizes have lower diversity (Frankham, 1996) and less adaptive potential (Hedrick, Robinson, Peterson, Vucetich, & Johnson, 2019). Our population genomic data support these expectations. Furthermore, Montezuma Quail exhibit lower levels of whole genomic heterozygosity than many other avian species (Fig. 3). The reduction of genomic diversity in Montezuma Quail is reflective of long-term declines in N_e over the last million years (Fig. 5B). More specifically, Montezuma Quail from Texas are the most genetically depauperate of the populations we surveyed with genomic diversity similar to vulnerable and endangered birds (Fig. 3B). Our Texas samples had genome-wide heterozygosity similar to raptors and other large birds (Table 1, Fig. 3B) even though small birds typically have more genetic diversity (Eo, Doyle, & DeWoody, 2011). Overall, we think the data reveal

428   that genomic erosion has likely reduced the evolutionary potential of Montezuma Quail in Texas and that this

429   reduction is unlikely to abate in the absence of gene flow through assisted translocation or other means.

430

431   *Isolation leads to more inbreeding*

432   A lack of migration among populations limits gene flow and accelerates inbreeding (Frankham, 1996;

433   Gong, Gu, & Zhang, 2010; Hedrick, Kardos, Peterson, & Vucetich, 2016; Keller, 2002; Madsen, Stille, & Shine,

434   1996; Pulanić et al., 2008). Our samples from Montezuma quail populations in the U.S. form independent genetic

435   clusters (Fig. 2C,D), which is unsurprising given the geographic distances among sampling sites and the limited

436   dispersal capacity of this ground-dwelling bird (Stromberg, 1990). These results are in general accordance with our

437   previous findings based on a small SNP panel (Mathur et al. 2019), but the divide in Arizona (Fig. 2C; Fig. S3) was

438   undetected with that same SNP panel. Our kinship analysis suggests that very few of our samples were derived from

439   related individuals (Fig. 2A), and our inbreeding estimates show that the Texas population is highly inbred as

440   compared to Arizona and New Mexico (Fig. 2B). Our samples were acquired opportunistically and that likely

441   reduced the probability of collecting related individuals. However, inbreeding itself can reduce estimates of kinship

442   as inbred individuals may have elevated number of alternate homozygous genotypes and a reduced number of

443   shared heterozygous genotypes (Waples et al., 2019). We observed an elevated incidence of alternative

444   homozygotes for within-Texas comparisons (Fig. S9) and we think the collective evidence shows that the small,

445   isolated population of Montezuma quail in West Texas is relatively inbred. This is key, as elevated inbreeding

446   means more of the potential genetic load will be realized (see below).

447

448   *Impact of genetic drift on local adaptation*

449   One of the major drivers of genetic erosion in small populations is genetic drift. In the absence of

450   migration, genetic drift can fix common alleles or lose rare alleles from the gene pool. Isolated populations with

451   historically low sizes can become phenotypically distinct over time (Holycross & Douglas, 2007; Schierup et al.,

452   2018) due to differences in nucleotide composition (D_{XY}) (Wakeley, 1996) or allele frequencies (F_{ST}) (Beaumont,

453   2005). The intensity of genetic differentiation due to drift is generally expected to be the same for all neutral loci in

454   the nuclear genome due to lack of selection pressures, but it is complicated by linked selection (McVean, Cai,

455   Macpherson, Sella, & Petrov, 2009; Rettelbach et al., 2019). Recent population genomic studies have shown that

456   different populations exhibit a heterogeneous differentiation landscape ("differentiation islands") across the genome

457   (Burri et al., 2015; Ellegren et al., 2012). We observe similar results in Montezuma Quail populations (Fig. 4; Fig.

458   S6) where many regions show highly significant values of F_{ST} even though global estimates seem biologically

459   insignificant (Table 2). Some of these high- F_{ST} windows no doubt represent statistical artefacts, but many of these

460   highly differentiated regions contain functional genes (Fig. 4) that could impact various fitness traits (Table S5) and

461   could be signatures of local adaptation (Willoughby et al., 2018). This idea is bolstered by our global estimates of

462   genic differentiation and the PBS results which suggest that coding genes are more rapidly diverging than the

463   genome overall (Fig. S8, Table 2). These signatures of adaptation analyses suggest that local adaptation could

464   constrain genetic rescue due to the possible reduction in fitness of interpopulation hybrids (Bell et al., 2019;

465   Whiteley, Fitzpatrick, Funk, & Tallmon, 2015). On the other hand, such analyses have the potential to identify

466   source populations that have adaptive genetic signatures most similar to the recipient population (e.g., Fig. S8) and

467   thus the greatest likelihood of success from a long-term, evolutionary perspective.

468

469   *The adaptive potential of small populations*

470   Understanding the adaptive response of a species to future environmental changes is a high priority for

471   conservation (Holderegger et al., 2019) as this response impacts the long-term probability of persistence (Hedrick et

472   al., 2019), but such an assessment is not straightforward. Genetic erosion is expected to affect adaptive potential by

473   either reducing the overall standing variation in adaptive regions or by the accumulation of deleterious mutations

474   (Lynch et al., 1995; Ohta, 1992). We evaluated these two detractors of adaptive capacity by considering variation

475   contained exclusively in genic regions and assessing their possible phenotypic impact. Montezuma Quail have over

476   17,000 genes and our results show that the nucleotide diversity in genic regions is lower relative to the whole

477   genomic background (Table 1). This is not entirely unexpected as most genes are highly conserved and most

478   mutations arising at these genes will be deleterious and subject to negative purifying selection (Rettelbach et al.,

479   2019). Despite reduced nucleotide diversity in genic regions, we observed higher levels of genic heterozygosity in

480   Montezuma Quail genes as compared to the entire genome (Fig. 3A). These results illustrate that populations can

481   maintain genic diversity, perhaps due to overdominance (e.g. Schou, Loeschcke, Bechsgaard, Schlötterer, and

482 Kristensen (2017), episodic diversifying selection on key fitness genes (Antonides, Mathur, & DeWoody, 2019;

483 Antonides, Mathur, Sundaram, Ricklefs, & DeWoody, 2019), and/or viability selection (Doyle et al., 2019). The

484 exact process or processes maintaining heterozygosity in genic regions is not clear, but the resulting pattern is

485 consistent with the myriad studies that have shown heterozygosity-fitness correlations in a wide variety of

486 eukaryotes (Mitton, 1997).

487   The proportion of deleterious mutations present in the genic regions should reflect the potential genetic

488 load (Charlesworth et al., 1993; Ellegren & Sheldon, 2008; Hedrick & Garcia-Dorado, 2016). Our results show

489 Arizona quail carry more high impact deleterious variants as compared to Texas quail and this difference tends to

490 diminish with variant impact (Fig. 6B). Most of the genic variants are non-coding (Fig. 6A) and thus do not impact

491 amino acid sequences but could impact expression levels (Harder, Willoughby, Ardren, & Christie, 2020). Recent

492 population genomics studies have shown via simulations (Coop et al., 2015) and empirical data (ÁVila, Amador, &

493 GarcÍA-Dorado, 2010; Do et al., 2015; Rettelbach et al., 2019) that most deleterious genic variants are eventually

494 culled by purifying selection but that small effect recessive mutations can persist in large populations.

495

496 *Conservation considerations*

497   Our results indicate that Montezuma quail populations in the U.S. exhibit low genomic diversity

498 comparable to a number of threatened and endangered species ((Brüniche-Olsen et al., 2019; de Villemereuil et al.,

499 2019; Zhan et al., 2013); Fig. 3B). Our genomic diversity estimates are consistent with predictions for small

500 declining populations, and we argue that our estimates of genic diversity better reflect the evolutionary potential of

501 the species. This study adds to the growing body of literature urging conservation organizations like IUCN to add

502 genetic diversity estimates as a consideration in the listing process (Allendorf, Hohenlohe, & Luikart, 2010;

503 Brüniche-Olsen, Kellner, Anderson, & DeWoody, 2018; Ralls et al., 2018; Willoughby et al., 2015)).

504   Theory suggests that deleterious mutations should be more abundant in small populations and empirical

505 data support this prediction for species like wooly mammoths (Barsh et al., 2017) and Iberian lynx (Abascal et al.,

506 2016), with critically low population sizes and ineffective purifying selection. However, most of the species that are

507 declining due to recent anthropogenic activities have maintained relatively large N_e with previous cycles of

508 bottlenecks and re-expansions, as in Montezuma Quail (Fig. 5B) and other birds (Nadachowska-Brzyska et al.,

509   2015). This study and another quantifying levels of genetic load across 42 mammalian species (van der Valk, de

510   Manuel, Marques-Bonet, & Guschanski, 2019) suggest that smaller populations have significantly lower proportions

511   of deleterious mutations whereas larger, more genetically diverse populations carry a higher burden of deleterious

512   mutations that contribute to genetic load. Our data indicate that large populations harbor a larger proportion of high

513   and low impact deleterious mutations in their genomes, but these variants are (on average) maintained at lower

514   frequencies and merely represent potential genetic load. In contrast, the realized genetic load impacts individual

515   fitness when these detrimental variants are homogenized due to inbreeding and/or drift. This pattern exists in part

516   because purifying selection against partially recessive deleterious recessive alleles is relaxed in large populations

517   where higher heterozygosity hides these alleles from selection. Thus, our genomic data illustrate and quantify the

518   incidence of potential genetic load in large populations (Arizona) relative to the realized genetic load in small,

519   inbred populations like Texas.

520

521

522   **Conclusions**

523   We analyzed whole genome sequences from different populations of Montezuma Quail in the U.S and

524   compared the relative impact of genetic erosion between populations of various sizes. Our results indicate that

525   Montezuma Quail populations in the U.S. have mean genome-wide heterozygosity comparable to other avian taxa of

526   conservation concern. We found that random drift due to isolation and higher inbreeding are the major driving force

527   behind these observed patterns of reduced genomic diversity. Most interestingly, our results reveal how small

528   populations are able to maintain adaptive potential by exhibiting higher genic heterozygosity despite a reduction in

529   overall genomic diversity. We find that larger populations carry a larger proportion of deleterious mutations

530   (potential genetic load) than small populations where recessive deleterious alleles are exposed to selection due to

531   inbreeding (realized genetic load). We think these data will be useful to those interested in the conservation of

532   Montezuma Quail, and that they illustrate the power of population genomics in evaluating adaptive potential in light

533   of fragmented landscapes and rapid environmental change.

534

535   **Acknowledgements**

545   **Data accessibility**

546   The sequence datasets generated during the current study are available in NCBI's Short Read Archive BioProject

547   accession # PRJNA623948, BioSample accession # SAMN14562436-509 and SRA accession # SRR11514056-129.

548   The scripts developed for analysis can be publicly accessed at https://github.com/samarth8392/MQU_PopGenomics

549

550   **Authors' contributions**

551   SM and JAD conceived and designed the research. SM performed all bioinformatic analyses. SM and JAD led the

552   writing effort.

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863 Table 1: Summary statistics for sequence coverage, inbreeding coefficients (F), per-site Watterson's theta (θ_w), heterozygosity (H) and effective population
864 sizes (N_E) for Montezuma quail populations analyzed in this study. The diversity indices were calculated for either the whole genome or just the genic
865 regions. N_E was calculated using an estimated genomic mutation rate of 3.14×10^{-9} with 95% CI calculated using standard error in θ_w estimates. Sequence
866 depth is measured in fold-coverage and breadth is measured as percentage of Montezuma quail assembly mapped by the reads.
867

| | N | Sequence depth (X) (mean ± SD) | Sequence breadth (%) (mean ± SD) | F (mean ± SD) | Whole genome | | Genic regions | | N_E (95% CI) |
					θ_w	H	θ_w	H	
Arizona	52	2.14 ± 0.78	69.45 ± 14.51	0.05 ± 0.08	5.37×10^{-4}	0.0020	4.89×10^{-4}	0.0044	42,795 (42,764 - 42,825)
Texas	15	1.45 ± 1.82	42.69 ± 30.17	0.33 ± 0.28	4.05×10^{-4}	0.0008	3.68×10^{-4}	0.0027	32,208 (32,182 - 32,234)
New Mexico	7	3.48 ± 1.78	84.16 ± 11.51	0.07 ± 0.08	5.57×10^{-4}	0.0011	3.49×10^{-4}	0.0023	36,417 (36,390 - 36,446)

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872 Table 2: Estimates of global F_{ST} between the different population pairs measured for either the whole genome or just the genic regions. 95% CI was
873 calculated using standard error in F_{ST} estimates by 100 bootstraps of 2D-SFS for each population pair.
874

| Population Pair | Mean Global F_{ST} (95% CI) | |
	Whole genome	Genic regions
Arizona - Texas	0.1287 (0.1286 - 0.12878)	0.2452 (0.2449 – 0.2455)
Texas - New Mexico	0.0962 (0.0961 - 0.0962)	0.1984 (0.1979 – 0.1986)
Arizona - New Mexico	0.0972 (0.0972 - 0.0973)	0.1926 (0.1925 – 0.1928)

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Figure 1: Montezuma Quail species range and sampling sites (from Mathur et al. 2019). Samples (N=60) were collected from the larger and most contiguous Arizona sites, from an intermediate-sized population in New Mexico (N=13 samples), and from a relatively isolated and small population in Texas (N=15 samples).

Figure 2: Inbreeding and population structure of Montezuma Quail. Samples analyzed in this study were mostly unrelated based on (A) kinship analysis. (B) Mean individual inbreeding co-efficient (F) was significantly higher in the Texas population with no significant difference between Arizona and New Mexico populations. Results from both (C) admixture and (D) PCA analysis clearly demarcate samples from the three collecting sites into independent genetic clusters. However, likelihood estimates indicate that most likely number of ancestral populations was K=4 (indicated with asterisk), where Arizona was divided into two subpopulations.

Figure 3: Estimated levels of heterozygosity in Montezuma quail. (A) Genic heterozygosity is higher than genome-wide heterozygosity in all three populations sampled. (B) Comparison of genome-wide heterozygosity with other birds indicates that smaller Montezuma Quail populations in Texas and New Mexico have genomic diversity comparable to vulnerable species (Brüniche-Olsen et al., 2019; de Villemereuil et al., 2019; Li et al., 2014). Heterozygosity was measured as the proportion of heterozygous genotypes in the whole genome.

Figure 4: Z-transformed F_{ST} estimates for comparisons made between Arizona and Texas Montezuma quail every 100 kb window (50 kb steps). The reads were mapped to the chicken genome and the windows were arranged according to chicken autosomal (1-33) or sex (Z, W) chromosomes. Scaffolds that were not part of the major chicken chromosomes were binned together as unplaced. We found many windows within each chromosome that had high (>5 SD) levels of differentiation and many of those windows contained genes with known function (red arrows). This shows that there is a very heterogeneous landscape of genetic differentiation within Montezuma quail genome and drift is most likely the evolutionary driver behind the observed patterns.

Figure 5: (A) Population trends and (B) demographic histories of Montezuma quail. Population trends indicate that only Arizona populations seems to be expanding (Fu's F < 0) whereas, both Texas and New Mexico populations are declining (Fu's F > 0). Error bars indicate 95% CI around the estimate. Montezuma quail experienced a strong historic bottleneck during the last glacial maxima (LGM) followed by re-expansion.

Figure 6: Larger populations have higher genetic load but smaller populations have higher inbreeding risks. (A) Schematic of eukaryotic gene structure and proportion of variants in different genic regions where the colors in each panel correspond to one another. Much of the genic variation exists outside the transcription unit. (B) Genetic load was estimated for each population as the proportion of deleterious mutations within annotated protein-coding genes. The Arizona samples had the highest proportions of high impact, moderate impact, and low impact variants consistent with their elevated level of genomic diversity (Fig. 3a). Note the difference in scales on y-axis. (C) Larger populations are more efficient at genetic purging via purifying selection. In all sized populations, the mean allele frequencies are smaller for deleterious mutations as compared to non-coding variants, but the small inbred Texas population has a higher frequency of negative impact variants than larger outbred Arizona or New Mexico populations. Error bars indicate 95% CI around the estimate. This pattern is likely because recessive deleterious alleles exist as heterozygotes in large populations and in homozygotes in small populations so that smaller populations have a higher risk of inbreeding depression despite having a smaller genetic load in terms of the proportion of compromised genes in the genome.