Genetic variability and population structure of the Montezuma quail (*Cyrtonyx montezumae*) in the northern limit of its distribution

Eduardo Sánchez-Murrieta [1], **Alberto Macías-Duarte** [Corresp., 2], **Reyna A Castillo-Gámez** [3], **Alejandro Varela-Romero** [3], **Angel B Montoya** [4], **Nohelia G Pacheco-Hoyos** [3]

[1] Maestría en Biociencias, Universidad de Sonora, Hermosillo, Sonora, Mexico

[2] Cuerpo Académico de Recursos Naturales, Universidad Estatal de Sonora, Hermosillo, Sonora, Mexico

[3] Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora, Hermosillo, Sonora, Mexico

[4] Partners for Fish and Wildlife Program, United States Fish and Wildlife Service, Las Cruces, New Mexico, United States of America

Corresponding Author: Alberto Macías-Duarte
Email address: alberto.macias@ues.mx

Low migration rate among populations may cause a decrease in genetic variation. Such is the case of the Montezuma quail (*Cyrtonyx montezumae*), a popular game bird with a limited flight capacity that prevents long-distance dispersal. In the northern limit of the species' distribution in Arizona, New Mexico, and Texas in the United States, the species inhabits oak savannas that are separated from one another by deserts. In this regard, the knowledge of genetic structure is likely to provide relevant guidelines for management. The objective of this study was to determine patterns of genetic variation in populations of the Montezuma quail using nine DNA microsatellites loci. We genotyped 119 individuals harvested from four populations: Arizona (AZ), West New Mexico (WNM), Central New Mexico (CNM) and West Texas (WTX). Montezuma quail populations had low observed heterozygosity (H_o = 0.22 ± 0.04) and a low number of alleles per locus (A = 2.41 ± 0.27) compared to other quail species. A global population genetic differentiation index R_{ST} of 0.045 suggests a weak genetic structure. Nevertheless, a Bayesian allocation analysis indicates that individuals were separated into three clusters (K = 3) placing the populations of Arizona and Texas in distinct clusters apart from other two populations of New Mexico. Despite being differentiated from each other, the Bayesian allocation analysis suggests admixture in the individuals between clusters, especially between populations of New Mexico and Arizona. Furthermore, an isolation-by-distance analysis indicates a strong correlation (R^2 = 0.84) and suggestive evidence (P = 0.08) of non-independence between geographical and genetic distances, though Moctezuma quail populations in the southwestern United States may not be completely isolated. Climate change projections indicate an increase in aridity conditions in this region, especially in temperate ecosystems

where the species occurs. In this scenario, corridors between the populations may disappear, thus causing their complete isolation.

Genetic variability and population structure of the Montezuma quail (*Cyrtonyx montezumae*) in the northern limit of its distribution

Eduardo Sánchez-Murrieta[1], Alberto Macías-Duarte[2], Reyna A. Castillo-Gámez[3], Alejandro Varela-Romero[3], Angel B. Montoya[4], & Nohelia G. Pacheco-Hoyos[3].

[1] Maestría en Biociencias, Universidad de Sonora, Hermosillo, Sonora, Mexico.

[2] Cuerpo Académico de Recursos Naturales, Universidad Estatal de Sonora, Hermosillo, Sonora, Mexico.

[3] Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora, Hermosillo, Sonora, Mexico.

[4] Partners for Fish and Wildlife Program, U.S. Fish and Wildlife Service, Las Cruces, New Mexico, U.S.A

Corresponding Author:

Alberto Macías Duarte

Av. Ley Federal del Trabajo S/N, Col. Apolo, Hermosillo, Sonora, 83100, Mexico

E-mail address: alberto.macias@ues.mx

Abstract

Low migration rate among populations may cause a decrease in genetic variation. Such is the case of the Montezuma quail (*Cyrtonyx montezumae*), a popular game bird with a limited flight capacity that prevents long-distance dispersal. In the northern limit of the species' distribution in Arizona, New Mexico, and Texas in the United States, the species inhabits oak savannas that are separated from one another by deserts. In this regard, the knowledge of genetic structure is likely to provide relevant guidelines for management. The objective of this study was to determine patterns of genetic variation in populations of the Montezuma quail using nine DNA microsatellites loci. We genotyped 119 individuals harvested from four populations: Arizona (AZ), West New Mexico (WNM), Central New Mexico (CNM) and West Texas (WTX). Montezuma quail populations had low observed heterozygosity (H_o = 0.22 ± 0.04) and a low number of alleles per locus (A = 2.41 ± 0.27) compared to other quail species. A global population genetic differentiation index R_{ST} = 0.045 suggests a weak genetic structure. Nevertheless, a Bayesian allocation analysis indicates that individuals were separated into three clusters (K = 3) placing the populations of Arizona and Texas in distinct clusters apart from other two populations of New Mexico. Despite being differentiated from each other, the Bayesian allocation analysis suggests admixture in the individuals between clusters, especially between populations of New Mexico and Arizona. Furthermore, an isolation-by-distance analysis indicates a strong correlation (R^2 = 0.84) and suggestive evidence (P = 0.08) of non-independence between geographical and genetic distances, though Moctezuma quail populations in the southwestern United States may not be completely isolated. Climate change projections indicate an increase in aridity conditions in this region, especially in temperate ecosystems where

45 the species occurs. In this scenario, corridors between the populations may disappear, thus
46 causing their complete isolation.

47 Introduction

48 A low migration rate between populations may cause a decrease in genetic variation (Frankham
49 1996), which can reduce ~~a species'~~ ability to adapt to environmental change (Allendorf and
50 Luikart, 2007). This reduced genetic variation can result in detriment to the metapopulations
51 long-term survival of the species (Arif & Khan 2008). Similarly, genetic variation loss in
52 populations reduces their viability by decreasing the average individual fitness (Reed &
53 Frankham 2003). Population size reductions in wildlife species has disrupted their geographical
54 distribution (Allendorf *et al*. 2008), increasing their isolation and reducing gene flow. Under this
55 metapopulation dynamics scenario, persistent harvest of game populations may further
56 exacerbate of the loss genetic variability (Harris *et al*. 2002). In this regard, the Montezuma quail
57 (*Cyrtonyx montezumae* Vigors 1830) is a popular game bird with limited flight capacity that
58 occupies habitat patches that are widely separated (Stromberg, 1990). The species inhabits arid
59 grasslands with the presence of different tree species, mainly oaks (*Quercus* spp.). The species is
60 distributed throughout the Western Sierra Madre from southern Arizona, New Mexico, and
61 Texas in the United States to Oaxaca and central Mexico. In New Mexico and Texas, the
62 Montezuma quail has a patchy distribution, ~~spatially separated from the rest of the species' range~~
63 ~~of distribution~~ (Stromberg *et al*, 2020).

64 The Montezuma quail is hunted ~~during the winter~~ in the states of Arizona and New
65 Mexico in the United States (Stromberg *et al,* 2020; Heffelfinger & Olding 2000). ~~In~~ Texas,
66 ~~Montezuma quail~~ populations are restricted to the Trans-Pecos region and the Edwards Plateau
67 (Albers & Gehlbach, 1990) ~~and there is no open hunting season for this species~~. In the mid-

1970s, a series of reintroductions began in different regions of Texas using individuals from Arizona, where the species attains its highest abundance in the United States (Wauer, 1973). Although these releases were performed out in places where the species was historically present, the success of these reintroductions has not been confirmed because the released individuals were not adequately monitored (Armstrong, 2006).

A population genetic survey is a convenient way to address the effect of isolation, hunting and reintroductions on the viability of Montezuma quail populations. Therefore, a greater knowledge of the species' population genetic structure and the possible consequences of this geographic arrangement are essential for better species' management. In this regard, the objective of this study was to determine the patterns of genetic variation of the among the Montezuma quail populations of Arizona, New Mexico and Texas using DNA microsatellite markers.

Materials & Methods

Tissue samples were extracted from specimens hunted in Arizona and New Mexico under numerous hunting licenses issued by Arizona Game and Fish Department and New Mexico Department of Game and Fish. Tissue samples from Texas originated from specimens collected under Scientific Permit Number SPR-0410-139 issued by Texas Parks and Wildlife Department. We assigned individual samples to four populations: Arizona (AZ), Central (CNM, east of the Río Grande), West New Mexico (WNM, west of the Río Grande) and West Texas (WTX). We obtained 119 samples: 32 from AZ, 36 from WNM, 26 from CNM and 25 from WTX (Figure 1). We used 25 mg of muscle tissue from the right wing of each individual to extract genomic DNA using a Qiagen® DNeasy Blood and Tissue extraction kit.

Twenty DNA microsatellite loci developed by Schable *et al*. (2004) for *Colinus virginianus* were evaluated. The choice of these was made based on heterozygosity and the number of alleles reported by the authors. For microsatellite amplification, 25 µL we prepared PCR reactions whose final concentrations were 2 µL of genomic DNA (with a concentration of 50/µL), 12.5 µL of MasterMix (GoTaq® Colorless Master Mix, Promega), 1 µL of each oligo and 8.5 µL of PCR water without endonucleases. Thermocycler conditions for the amplification were modified according to the specifications of the manufacturer of the MasterMix used and the alignment temperatures of the oligos, which varied between 45 °C and 57 °C. The program used was as follows: a cycle of 95 °C for 2 minutes; five cycles of 94 ° C for 30 seconds, 45 °C for 30 seconds and 72 °C for 30 seconds; then 35 cycles of 94 °C for 45 seconds, 45 °C for 45 seconds and 72 °C for one minute; followed by a cycle of 72 °C for two minutes. Ten microsatellite loci with the highest polymorphism and concordance in the fragment size as reported by the author were chosen. Selected loci were marked with fluorophores in the sequence 5'-3'. A post-PCR multiplex array for fragment reading was performed, this was performed on an ABI 3730xl sequencer (Applied Biosystems) of Macrogen.

Allele scoring was conducted using program GeneMarker v. 2.6.4 (Hulce *et al*., 2011). Loss of alleles, null alleles and excess homozygotes were assessed using program Micro-Checker v 2.2.3 (Van Oosterhout *et al*., 2004). Genetic variability estimator's locus alleles (*A*) expected heterozygosity (H_e) and observed heterozygosity (H_o) were obtained for each locus and population using GenAlEx v 6.5 (Smouse and Peakall 2012). Using the Arlequin v 3.5 software (Excoffier & Lischer 2010), possible deviations from the Hardy-Weinberg equilibrium (HWE), were calculated for each locus in each population.

Population genetic differentiation index R_{ST} was calculated using Arlequin v 3.5 (Excoffier & Lischer 2010) to determine the degree of genetic differentiation among populations. Population structure analysis was conducted using software Structure (Pritchard *et al*. 2000) which estimates the most probable number of clusters or groups (K) that exist within the analyzed population. Parameters established for the analysis were 10,000 burnins, 50,000 repetitions of Monte Carlo Markov Chains, 25 iterations, a K value (number of clusters) between 1-4, an ancestral mixing model and a correlated allelic frequency model. Outputs from program Structure were analyzed using the method of Evanno *et al.* (2005) as implemented by software Structure Harvester (Earl 2012) to determine the most probable number of genetic clusters. This method estimates Delta K (ΔK), which is the difference between the values of the logarithmic likelihood of each analysis iteration for the four clusters.

A Mantel test was conducted using program of Genepop v 4.3 (Rousset 2014) to determine if there is a distance isolation pattern between the populations of the Montezuma quail. Statistic R_{ST} was used as a measure of genetic distance between populations. We performed 10,000 permutations to estimate the statistical significance ($P < 0.05$) of the null hypothesis of independence between genetic and geographic distance.

Results

All samples ($n = 119$) were analyzed using 10 microsatellite loci. However, ~~some individuals did not show amplification~~ in loci Quail 3, Quail 13, Quail 24 and Quail 27. For the first three loci, only an individual did not amplify in CNM, WNM, and AZ respectively. For locus Quail 27, 3 individuals of CNM and 2 of WNM showed no alleles. For locus Quail 41, despite having visualized the amplification of fragments in agarose gels, it was only possible to genotype 21 individuals and this locus was excluded from the analysis. The presence of null alleles was

detected in loci Quail 27 and Quail 44 and were therefore excluded from the analyses of genetic structure (see below). Seven loci were polymorphic in at least one population, while loci Quail 25 and Quail 44 were monomorphic for all populations (Table 1). We found exclusive alleles for loci Quail 03, Quail 13, Quail 27, and Quail 31 at populations AZ, CNM and WTX. Locus Quail 27 deviated from the HWE in AZ, CNM and WNM, and had heterozygous deficit. Locus Quail 31 also deviated from the HWE in all populations, with an excess of heterozygotes in AZ and deficit of heterozygote in the other three populations. Locus Quail 24 did not meet the HWE at WNM, where many of the individuals presented the same homozygous genotype.

Overall estimators of genetic variability had the following values: number of alleles 2.41 ± 0.27 per locus (range = 1 – 11), mean observed heterozygosity of 0.22 ± 0.04 and mean expected heterozygosity of 0.24 ± 0.04 (Table 1). Genetic variability estimates remained similar among all populations. CNM had the highest values of observed heterozygosity (0.25 ± 0.08), followed by WTX (0.23 ± 0.08), and AZ (0.22 ± 0.10), while the WNM population had the lowest values (0.19 ± 0.07).

The global population genetic differentiation was low (R_{ST} = 0.045). However, pairwise differentiation between AZ–WTX (R_{ST} = 0.094. P = 0.001) and AZ–CNM (R_{ST} = 0.061. P = 0.009) were statistically significant (Table 2). Similarly, the differentiation between WTX and WNM had statistical significance (R_{ST} = 0.094, P = 0.001) (Table 2). We found 3 genetic clusters (K = 3) as determined by the method of Evanno *et al*. (2005) (Figure 2): individuals from WNM and CNM were assigned to one cluster, while AZ and WTX are located each in two separates clusters (Figure 3).

The Mantel test found a strong correlation (R^2 = 0.84) between geographical distance and genetic distance (R_{ST}) across populations (Figure 4). Nevertheless, we found only suggestive

158 evidence ($P = 0.08$) of non-independence between geographical distance and genetic distance.
159 According to R_{ST} statistics, AZ and WTX populations had the greatest genetic and geographic
160 distances while CNM and WNM populations had a shorter genetic and geographical distance.

161 Discussion

162 Montezuma quail observed heterozygosity and number of alleles per locus obtained in this study
163 ($H_o = 0.22 \pm 0.02$; $A = 2.41 \pm 0.27$) indicate the population's low genetic variability in the
164 southwestern United States. Similarly, Mathur *et al* (2019) reported low levels of genetic
165 variability in the Montezuma quail using molecular markers (SNP), with $H_o = 0.32 \pm 0.17$. In
166 Odontophoridae, observed heterozygosity was similar and even higher than those reported by the
167 literature using microsatellite loci (Hale & Hughes 2003, Terhune 2008, Orange *et al*. 2014).
168 Low genetic variability observed on Montezuma quail populations may be caused by drift acting
169 on small and isolated populations under a patchy distribution at the northern limit of the species,
170 i.e. oak savanna islands separated by vast desert grasslands. In this regard, Lesica & Allendorf
171 (1995) mention that the low migration rates between the peripheral populations and those who
172 are closer to the center of the species geographic range, could promote a gene drift effect, hence,
173 fixed alleles and reduction of genetic variability are more likely to occur.

174 The low R_{st} observed value suggests a weak population structure. In addition, pairwise R_{st}
175 values indicate a low to moderate differentiation in some populations. For instance, AZ
176 population is different from CNM ($R_{ST} = 0.061$; $P = 0.011$) and WTX ($R_{st} = 0.094$; $P = 0.001$)
177 populations. In addition, AZ population is no differentiated from WNM ($R_{ST} = 0.014$; $P = 0.054$),
178 suggesting an extant corridor for Montezuma quail these two populations. The Tonto National
179 Forest in central Arizona is connected by mountain ranges to the south of the state and represents
180 the northern limit of the Montezuma quail's geographic distribution. Sightings of the species in

Tonto National Forest suggest a persistent presence in this area (Sullivan *et al*. 2009) and potential habitat for the species in Tonto National Forest. This area is also connected by seemingly Montezuma quail habitat to Apache–Sitgreaves National Forest and Gila National Forest in New Mexico, between AZ and WNM. Furthermore, a radiotelemetry study conducted in Arizona found that individuals of Montezuma quail move more than 2 kilometers (Chavarría, 2017). We cannot rule out the species presence in seemingly unoccupied areas since the reported closest distance between sightings in the two national parks in Arizona were close to 20 km (Sullivan *et al*., 2009), and they are difficult to detect because of the hiding strategy (remain still to be confused with the floor using its cryptic plumage).

Furthermore, the lowest rate of populations genetic differentiation occurred between WNM and CNM (R_{ST} = 0.014; P = 0.063). Individuals were collected from these populations with the smallest geographical distance between them, thus it is likely that the exchange of individuals among these populations is highest compared to other populations. Although some CNM individuals appear to be geographically isolated from the rest of the population (for example, individuals collected in the Lincoln National Forest), Montezuma quail can occur in areas with riparian vegetation nearby oak forest (Stromberg *et al*, 2020), hence, they may be using riparian corridors as transit between the WNM and CNM populations. In addition, their sightings in Rio Grande do support this statement (Sullivan *et al*., 2009).

The differentiation R_{ST} values between AZ and WTX (R_{ST} = 0.094; P = 0.001) and WTX–WNM populations (R_{ST} = 0.059; P = 0.013) were statistically significant and the highest among all pairwise comparisons. In contrast, the R_{ST} value among WTX–CNM populations (R_{ST} = 0.033; P = 0.096) was not significant, an indicator of genetic flow between these populations. Greene et al. (2020) reported movements of up to 12 km for individuals of the Montezuma quail

in this Texas region, specifically in Davis Mountains. This area is approximately 20 km away from the Guadalupe Mountains, which is near the Texas–New Mexico state line, thus representing a possible route of exchange between the two populations. However, the existence of desert patches between the two mountainous areas and the highest R_{ST} value compared to populations which also showed no difference between them, postulates that the possible migration between populations should be taken with caution. The Bayesian assignment method assigned Montezuma quail populations in the southwestern United States to three clusters: AZ, WNM–CNM, WTX. However, mixing existed between clusters were evident in New Mexico populations. Bayesian assignment by Mathur *et al*. (2019) did not detect separation between Arizona and New Mexico using genomic-wide single nucleotide polymorphism as we did, possibly due to an unbalanced sample size (5 individual samples from New Mexico, 16 from Texas and 165 for Arizona). Nevertheless, the results obtained in both works were similar regarding the low differentiation between the populations of AZ–WNM and WNM–CNM. Thus, these populations would be representing a single cluster, while the WTX population would be isolated from the rest. In this regard, approximately 40% of individuals assigned to the WTX population came from the New Mexico cluster, suggesting migration between groups or insufficient time for differentiation. Results from this work support the idea that the Montezuma quail is a "ring species", where AZ and WTX populations were colonized by divergent populations dispersing northbound from the ancestral population in central Mexico through the Sierra Madre Occidental and Sierra Madre Oriental, respectively (Mathur *et al.*, 2023).

The Mantel test showed suggestive evidence of isolation by distance in the Montezuma quail populations. The change in temperature and precipitation patterns apparently expanded in altitude and latitude boundaries of deserts surrounding the Montezuma quail habitat in the

southwestern United States. (Archer and Predick 2008, Seager *et al*. 2007; Williams *et al*. 2010). In this context, constant fragmentation and changes in the extent of habitats occupied by these species can promote a decrease in transit areas between populations, causing complete separation of populations, increasing the risk of survival of the species in the medium and long term.

Conclusions

The genetic structure detected for Montezuma quail populations is valuable for the future management of this charismatic bird under harvest. Our results indicate low levels of genetic variability due to low values of numbers of alleles per locus and observed heterozygosity in the four populations analyzed. There is a weak genetic structure among the three clusters according to the Bayesian population analysis: Arizona, West New Mexico–Central New Mexico, and West Texas. Despite the differentiation, the analysis suggests mixing between populations, which may indicate migration, especially between New Mexico and Arizona populations. Migration between New Mexico and West Texas is also possible. In this context, our results support isolation by distance between populations, due to the non-independence between geographical distance and genetic distance. Despite this, Montezuma quail populations in the southwestern United States may not fully isolated, despite habitat loss and fragmentation in this region. Corridors may open or close periodically depending on annual precipitation.

Acknowledgements

We thank Arizona Quail Alliance, New Mexico Quail, Southern Arizona Quail Forever, Texas Parks and Wildlife Department, The Timken Foundation, and Pheasants Forever and Quail Forever in Texas for their financial support. We thank Robert Perez of the Texas Parks and Wildlife Department for his logistical and financial support that made the collection of Montezuma quail specimens in Texas possible. We also thank the volunteer hunters who

collected or assisted collecting specimens including May Dennis, Randy Gray, Steve Hopkins, Dennis Kavanagh, Mike Sullins, Ray Trejo, Bill Miller, James Weaver, and numerous anonymous quail hunters.

References

Albers, R.P. & F.R. Gehlbach. 1990. Choices of feeding habitat by relict Montezuma quail in central Texas. *Wilson Bull.* 102:300-308.

Allendorf, F. W., P.R. England, G. Luikart, P.A. Ritchie, & N. Ryman. 2008. Genetic effects of harvest on wild animal populations. *Trends Ecol. Evol.* 23(6):327-337.

Allendorf, F. W. & G. Luikart. 2007. Conservation and the Genetics of Populations. Blackwell Publishing. Oxford, England.

Archer, S.R. & K.I. Predick. 2008. Climate change and ecosystems of the southwestern United States. *Rangelands J.* 30, 23-29.

Arif, I.A. & H.A. Khan. 2009. Molecular markers for biodiversity analysis of wildlife animals: a brief review. *Anim. Biodiv. and Conserv*. 32:9-17.

Armstrong, K. 2006. Texas quails: ecology and management. Texas A&M University Press. Texas, United States of America.

BirdLife International. 2016. *Cyrtonyx montezumae*. The IUCN Red List of Threatened Species 2016:e.T22728212A94975065. http://dx.doi.org/10.2305/IUCN.UK.20163.RLTS.T22728212A94975065.en. Downloaded on 11 December 2017.

Chavarria, P. M., N. J. Silvy, R.R. Lopez, D. S. Davis, & A. Montoya. 2017. Ranges and movements of Montezuma quail in southeast Arizona. In National Quail Symposium Proceedings, 8:94.

272 Earl, D. A. 2012. STRUCTURE HARVESTER: a website and program for visualizing
273 STRUCTURE output and implementing the Evanno method. *Conserv. Genet.*
274 *Resour.* 4:359-361.
275 Evanno, G., S. Regnaut, & J. Goudet. 2005. Detecting the number of clusters of individuals
276 using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14:2611-2620.
277 Excoffier, L. & H.E. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform
278 population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10:564-567.
279 Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. *Conserv.*
280 *Biol.* 10:1500-1508.
281 Greene, C. D., Harveson, L. A., Cross, J. G., & Luna, R. S. 2020. Spatial ecology and habitat
282 utilization of Montezuma quail in the Davis mountains of Texas. *Texas J. Sci.* 72(1),
283 Article-4.
284 Hale, A. M. & C. R. Hughes. 2003. Characterization of polymorphic microsatellite loci in a
285 neotropical wood-quail, *Odontophorus leucolaemus*. Mol. Ecol. Resour. 3:508-510.
286 Harris, R. B., W. A. Wall, & F. W. Allendorf. 2002. Genetic consequences of hunting: what do
287 we know and what should we do? *Wildlife Soc. B.* 30:634-643.
288 Heffelfinger, J. R. & R. J. Olding. 2000. Montezuma quail management in Arizona. In National
289 Quail Symposium Proceedings 4:45.
290 Hulce, D., X. Li, T. Snyder-Leiby, & C. J. Liu. 2011. GeneMarker® genotyping software: Tools
291 to increase the statistical power of DNA fragment analysis. *JBT* 22(Suppl), S35.
292 Lesica, P. & F.W. Allendorf. 1995. When are peripheral populations valuable for
293 conservation? *Conserv. Biol.* 9:753-760.

294 Mathur, S., J. M. Tomeček, A. Heniff, R. Luna, & J. A. DeWoody. 2019. Evidence of genetic
295 erosion in a peripheral population of a North American game bird: the Montezuma quail
296 (*Cyrtonyx montezumae*). *Conserv. Gen.* 20:1-13.
297 Mathur, S., J. M. Tomeček, L. A. Tarango-Arámbula, R. M. Perez, & J. A. DeWoody. 2023. An
298 evolutionary perspective on genetic load in small, isolated populations as informed by
299 whole genome resequencing and forward-time simulations. Evolution 77:690–704.
300 Orange, J. P., C. A. Davis., R.A. Van Den Bussche., S. D. Fuhlendorf., D. R. Elmore., E. T.
301 Thacker, & M. E. Judkins. 2014. Development and characterization of 23 microsatellite
302 loci for scaled quail (*Callipepla squamata*). *Conserv. Genet. Resour.* 6:929-931.
303 Pritchard, J. K., M. Stephens, & P. Donnelly. 2000. Inference of population structure using
304 multilocus genotype data. *Genetics* 155:945-959.
305 Reed, D. H. & R. Frankham. 2003. Correlation between fitness and genetic diversity. *Conserv.*
306 *Biol.* 17:230-237.
307 Rousset, F. 2014. Genepop 4.3 for Windows/Linux/Mac OS X. This documentation: July, 8,
308 2014.
309 Schable, N. A., B. C. Faircloth, W. E. Palmer, J. P. Carroll, L. W. Burger, L. A. Brennan, C.
310 Hagen, & T. Glenn. 2004. Tetranucleotide and dinucleotide microsatellite loci from the
311 northern bobwhite (*Colinus virginianus*). *Mol. Ecol. Notes* 4:415–419.
312 Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H.P. Huang, N. Harnik, A. Leetma,
313 N. C. Lau, C. Li, J. Velez, & N. Naik. 2007. Model projections of an imminent transition to
314 a more arid climate in southwestern North America. *Science* 316:1181-1184.
315 Smouse, R. P. P. & R. Peakall. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic
316 software for teaching and research—an update. *Bioinformatics* 28:2537-2539.

317 Stromberg, M. R. 1990. Habitat, movements and roost characteristics of Montezuma quail in
318 southeastern Arizona. *Condor* 92:229-236.
319 Stromberg, M. R., A. B. Montoya, & D. Holdermann. 2020. Montezuma Quail (*Cyrtonyx*
320 *montezumae*), version 1.0. In Birds of the World (P. G. Rodewald, Editor). Cornell Lab of
321 Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.monqua.01
322 Sullivan, B. L., C. L. Wood, M. J. Iliff, R. E. Bonney, D. Fink, & S. Kelling. 2009. eBird: a
323 citizen-based bird observation network in the biological sciences. *Biol. Conserv*. 142:
324 2282-2292.
325 Terhune, T. M. 2008. Effects of translocation on population genetics and demographics of a
326 northern bobwhite (*Colinus virgnianus*) population among a fragmented landscape in
327 southwestern Georgia. Doctoral dissertation. University of Georgia.
328 Van Oosterhout, C., W. F. Hutchinson., D. P. Wills. & P. Shipley. 2004. MICRO-CHECKER:
329 software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol.*
330 *Resour.* 4(3), 535-538.
331 Wauer, R. H. 1973. Birds of Big Bend National Park and vicinity. University of Texas Press.
332 Texas, United States of America.
333 Williams, A.P., C. D. Allen, C. I. Millar, T. W. Swetnam, J. Michaelsen, C. J. Still, & S. W.
334 Leavitt. 2010. Forest responses to increasing aridity and warmth in the southwestern
335 United States. *P. Natl. Acad. Sci. USA* 107:21289-21294.

Figure 1

Sampling locations for Montezuma quail.

The gray area represents the estimated geographic distribution of the Montezuma quail (BirdLife International 2016). Circles, squares, triangles, and diamonds represent individuals from the populations of Arizona, West New Mexico, Central New Mexico and West Texas, respectively.

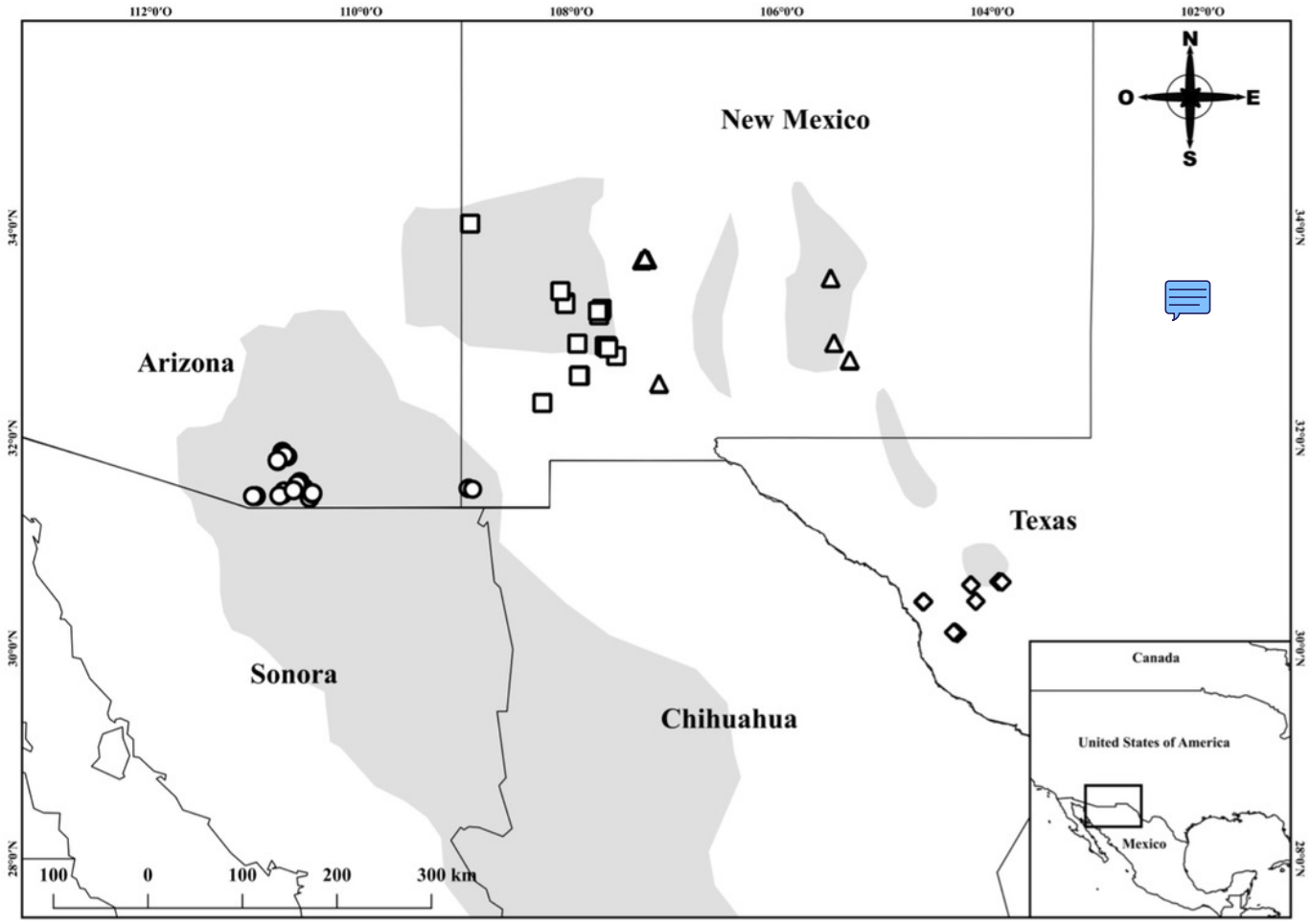

Figure 2

Log-likelihood values (Log prob) vs. number of groups from the Bayesian method obtained in STRUCTURE (Δ*K*).

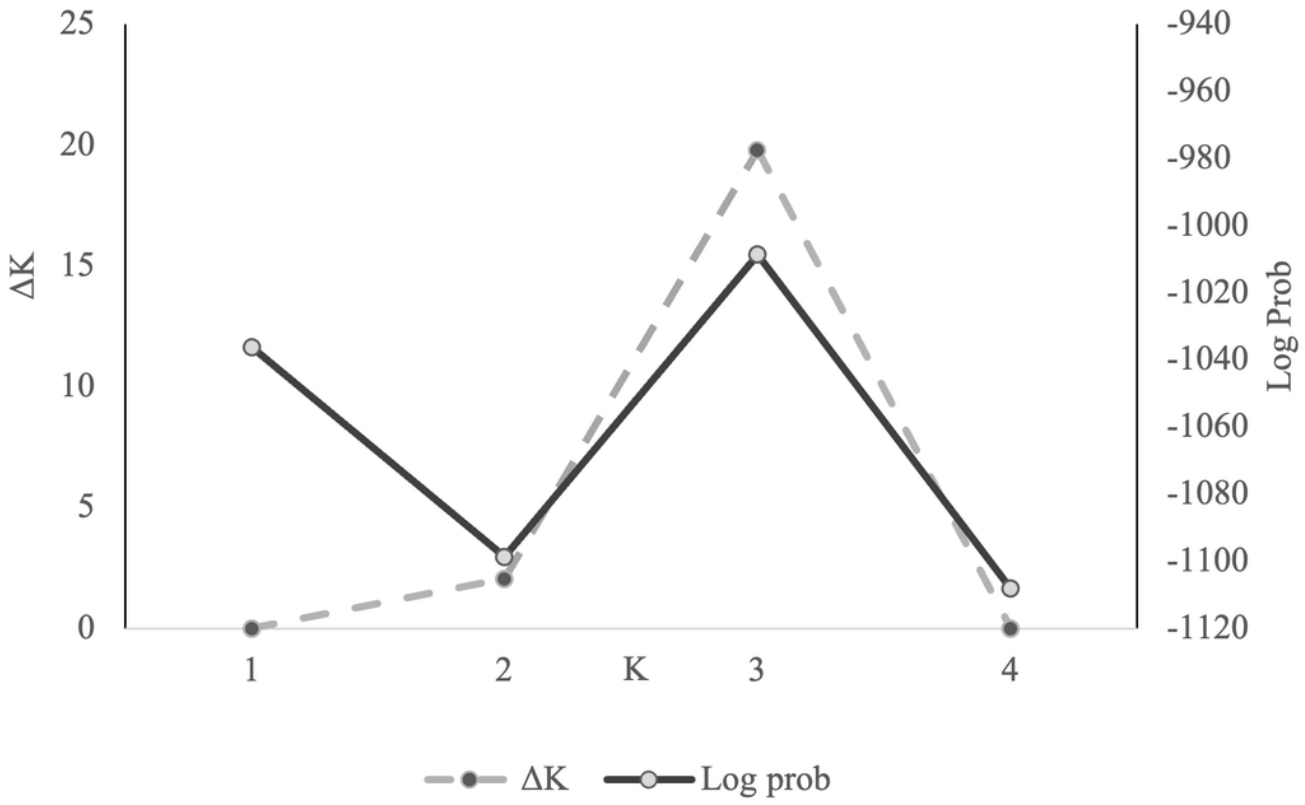

Figure 3

Geographic variation among Montezuma quail populations in the posterior probability of membership to each of the 3 populations inferred by program *STRUCTURE*.

Each pie chart represents ancestry estimates for individuals of each population based on K = 3 genetic clusters (Arizona, New Mexico and Texas) . Colors green, blue and red correspond to individuals with affinity to the population of Arizona, New Mexico and Texas, respectively.

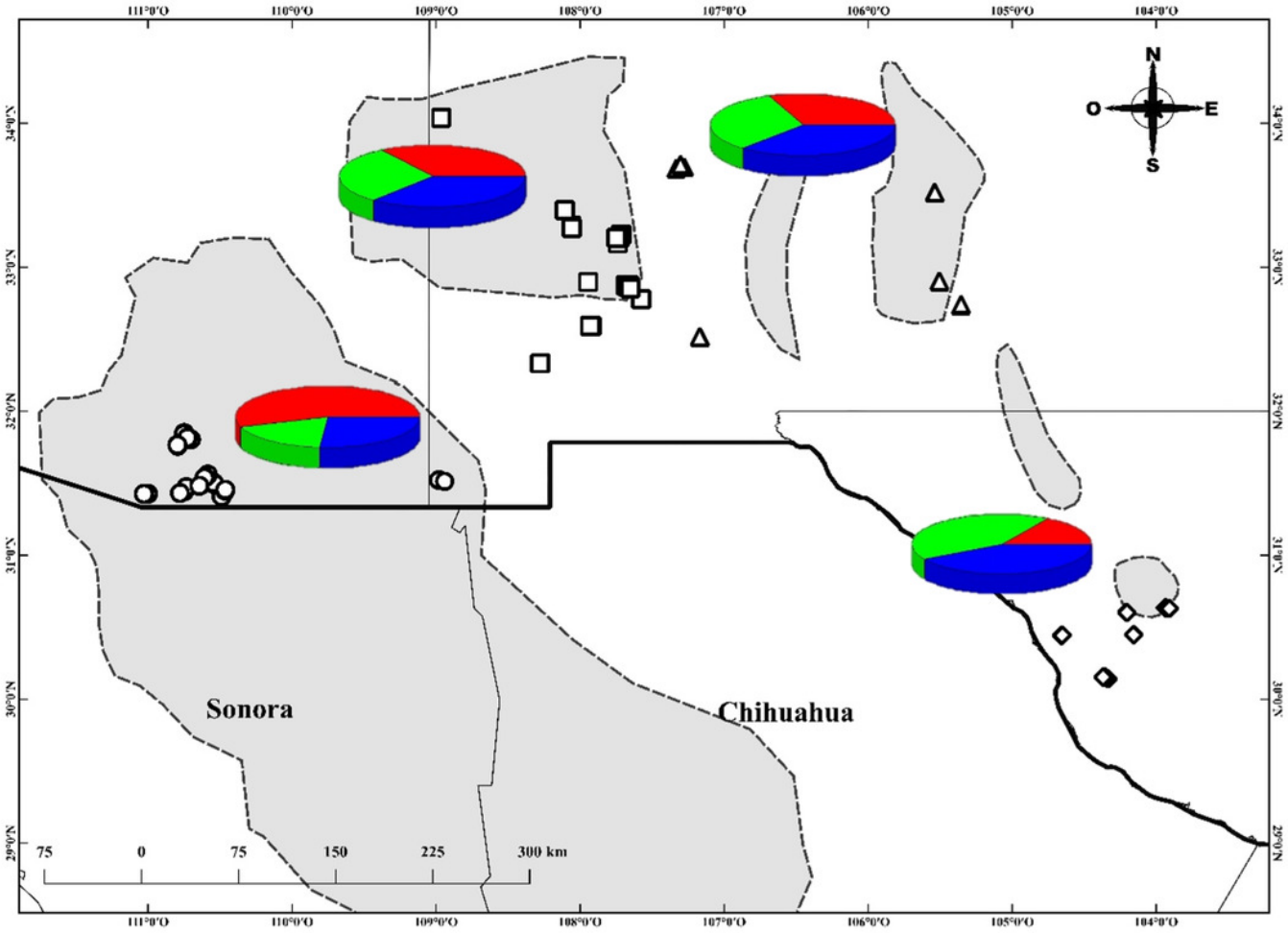

Figure 4

Correlation between geographical distance and the RST statistic values for each pair of populations (Mantel test $R^2 = 0.88$, $P = 0.03$).

The letters on the graph indicate each pairwise population comparison: a = CNM-WNM, b = AZ-WNM, c = CNM-WTX, d = AZ-CNM, e = WNM-TX and f = AZ-WTX.

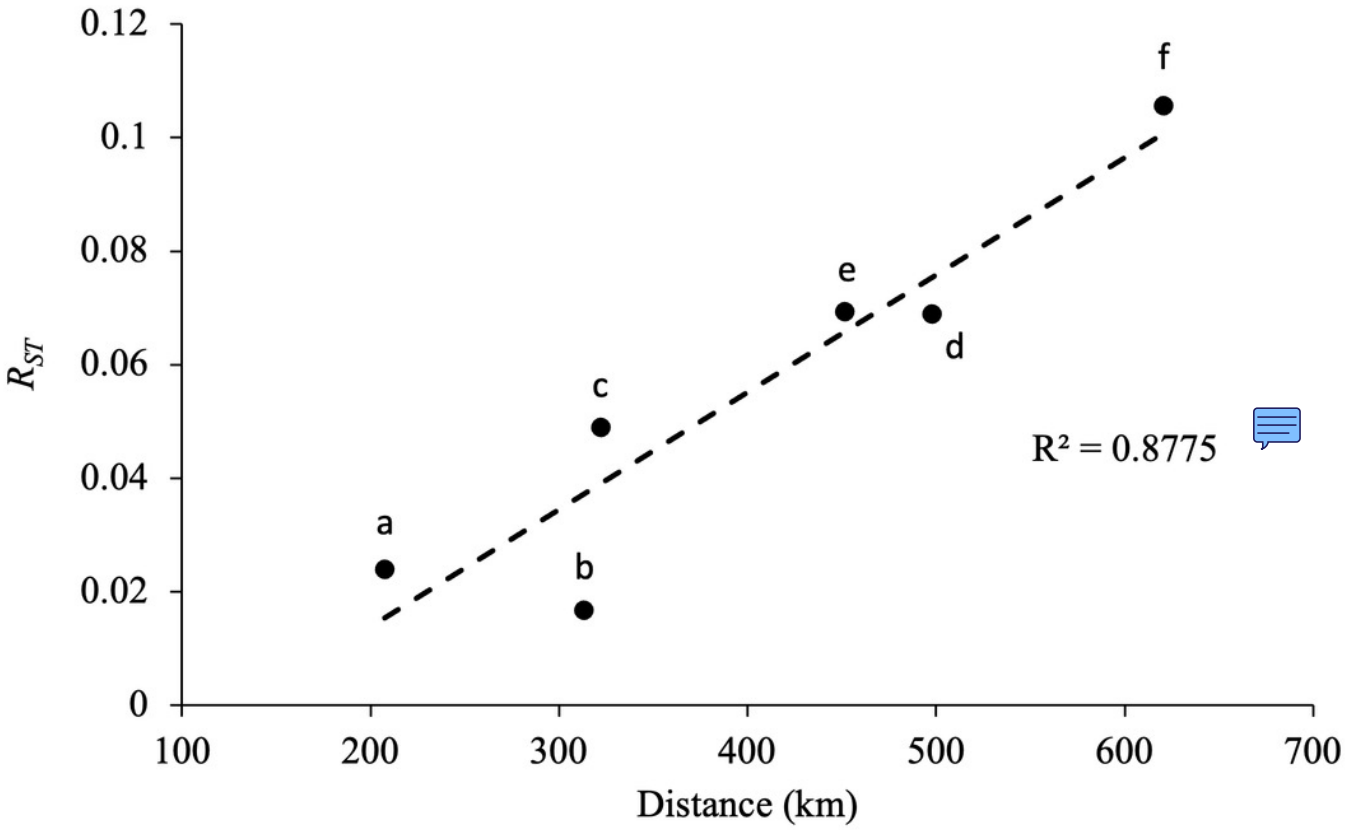

Table 1(on next page)

Genetic variability estimators for nine microsatellite loci in Montezuma quail populations of Arizona, Central New Mexico, West New Mexico and West Texas.

Symbols N, A, H_o and H_e denote sample size, number of alleles (exclusive alleles), observed heterozygosity and expected heterozygosity, respectively. Numbers marked in bold correspond to loci that deviated from Hardy-Weinberg equilibrium under Fisher's exact test ($P < 0.05$).

	Arizona				Central New Mexico				West New Mexico				West Texas			
Locus	N	*A*	H_o	H_e	N	*A*	H_o	H_e	N	*A*	H_o	H_e	N	*A*	H_o	H_e
Quail 03	32	2	0.16	0.2	23	3(1)	0.22	0.20	36	3	0.22	0.20	25	2	0.12	0.11
Quail 09	32	1	0.00	0.00	24	2	0.13	0.12	36	2	0.17	0.15	25	1	0.00	0.00
Quail 13	32	1	0.00	0.00	24	1	0.00	0.00	35	1	0.00	0.00	25	2(1)	0.04	0.04
Quail 14	32	2	0.41	0.33	24	2	0.58	0.50	36	2	0.47	0.49	25	2	0.56	0.50
Quail 24	31	3	0.42	0.35	24	3	0.42	0.40	**36**	**2**	**0.03**	**0.08**	25	3	0.36	0.30
Quail 25	32	1	0.00	0.00	24	1	0.00	0.00	36	1	0.00	0.00	25	1	0.00	0.00
Quail 27	**32**	**3**	**0.19**	**0.45**	**22**	**4(2)**	**0.27**	**0.61**	**33**	**2**	**0.21**	**0.5**	25	4(1)	0.52	0.62
Quail 31	**32**	**5(1)**	**0.84**	**0.63**	**24**	**7(1)**	**0.63**	**0.77**	**36**	**6**	**0.58**	**0.68**	**25**	**7(3)**	**0.48**	**0.69**
Quail 44	32	1	0.00	0.00	24	1	0.00	0.00	36	1	0.00	0.00	25	1	0.00	0.00
Mean	31.89	2.11	0.22	0.22	23.89	2.67	0.25	0.29	35.56	2.22	0.19	0.23	25.00	2.56	0.23	0.25
S.E.	0.11	0.45	0.10	0.08	0.35	0.65	0.08	0.10	0.34	0.52	0.07	0.09	0.00	0.65	0.08	0.09

1

Table 2 (on next page)

Genetic differentiation index values R_{ST} (below the diagonal) between Montezuma quail populations in Arizona (AZ), Central New Mexico (CNM), West New Mexico (WNM) and West Texas (WTX).

Numbers above the diagonal represent *P*-values for each pairwise population comparison from an exact Fisher test. The index R_{ST} values that were statistically significant are marked in bold: (*) significant ($P < 0.01$), (**) highly significant ($P < 0.001$)

1

	AZ	CNM	WNM	WTX
AZ	-	**0.009**	0.054	**0.001**
CNM	**0.061***	-	0.063	0.072
WNM	0.014	0.021	-	**0.009**
WTX	**0.094****	0.041	**0.059***	-

2