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], **James H Weaver** [5], **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

[5] Texas Parks and Wildlife Department, Fort Davis, Texas, United States of America

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

Restricted movement among populations decreases genetic variation, which may be the case for the Montezuma quail (*Cyrtonyx montezumae*), a small game bird that generally does not fly long distances. In the northern limit of the species' distribution the species inhabits the oak-juniper-pine savannas of Arizona, New Mexico, and Texas. Thus, understanding genetic structure can provide relevant management guidelines. The objective of this study was to determine patterns of genetic variation in Montezuma quail populations using nine DNA microsatellite loci. We genotyped 119 individuals from four study populations: Arizona, Western New Mexico, Central New Mexico, and West Texas. Compared to other quail, heterozygosity was low (H_o = 0.22 ± 0.04) and there were fewer alleles per locus (A = 2.41 ± 0.27). The global population genetic differentiation index R_{ST} = 0.045 suggests little genetic structure, even though a Bayesian allocation analysis suggested three genetic clusters (K = 3). This analysis also suggested admixture between clusters. Nevertheless, 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. Climate change projections indicate an increase in aridity for 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], James H. Weaver[5] , and 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

[5] Texas Parks and Wildlife Department, Fort Davis, Texas, 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

Restricted movement among populations decreases genetic variation, which may be the case for the Montezuma quail (*Cyrtonyx montezumae*), a small game bird that ~~generally does not fly~~ long distances. In the northern limit of ~~the species'~~ distribution ~~the species~~ inhabits ~~the~~ oak-juniper-pine savannas of Arizona, New Mexico, and Texas. ~~Thus,~~ understanding genetic structure can provide relevant management guidelines. The objective of this study was to determine patterns of genetic variation in Montezuma quail populations using nine DNA microsatellite loci. We genotyped 119 individuals from four study populations: Arizona, Western New Mexico, Central New Mexico, and West Texas. Compared to other quail, heterozygosity was low ($\overline{H}_O = 0.22 \pm 0.04$) and there were fewer alleles per locus ($\overline{A} = 2.41 \pm 0.27$). The global population genetic differentiation index $R_{ST} = 0.045$ suggests little genetic structure, even though a Bayesian allocation analysis suggested three genetic clusters ($K = 3$). This analysis also suggested admixture between clusters. Nevertheless, 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. Climate change projections indicate an increase in aridity for this region, especially in temperate ecosystems where the species occurs. In this scenario, corridors between the populations may disappear, thus causing their complete isolation.

Introduction

Dispersal influences geographic patterns of genetic variation (Waters *et al.*, 2020). If a species' mobility is small compared to the distance between disjoint populations, restricted movements may reduce local genetic variation (Frankham, 1996). ~~This may~~ reduce the ability of disjoint populations to adapt to environmental change (Allendorf & Luikart, 2007), and may result in a detriment ~~to the metapopulation's long-term survival~~ (Arif & Khan, 2009). Similarly, genetic

variation loss reduces ~~a population's~~ viability by decreasing ~~the~~ average individual fitness (Reed & Frankham, 2003). Population size reductions in wildlife species ~~under harvest~~ have disrupted their geographical distribution, increased their isolation, and reduced gene flow (Allendorf *et al.* 2008). Under this metapopulation dynamics scenario, persistent harvest may further exacerbate the loss of genetic variability (Harris *et al.*, 2002). The Montezuma quail (*Cyrtonyx montezumae* Vigors 1830) is a small game bird with limited ~~flight~~, that has a naturally ~~disjoint~~ geographic distribution. Montezuma quail (Montezumas hereafter) inhabit temperate woodlands and savannas associated with mountains ranges in southeastern Arizona, southern ~~half of~~ New Mexico ~~southern half~~, and west Texas in the United States (Stromberg *et al.*, 2020). The Montezuma's geographic distribution extends southwards into Mexico along the Sierra Madre Occidental and ~~Sierra Madre~~ Oriental to the Trans-Mexican Volcanic Belt and the Sierra Madre del Sur. Montezumas ~~frequently~~ occupy habitat patches in isolated mountain ranges that are widely separated by unsuitable arid lands (Stromberg *et al.*, 2020). Dense grass is a necessary habitat component for cover (Brown, 1979), nesting (Bishop, 1964), and ~~harboring plant food items~~ (López-Bujanda *et al.*, 2022). Montezumas are sedentary, with home range sizes averaging 50 ha (Chavarría *et al*., 2017). The Montezuma's nesting season ~~extends~~ from June to October, ~~whose phenology~~ is largely influenced by summer precipitation. Females produce a single clutch of around 10 eggs (Stromberg *et al.*, 2020). Montezumas live in coveys during the non-breeding season, but form pairs for nesting by as early as late February. The species feeds primarily on underground plant structures, such as bulbs and tubers of woodsorrel (*Oxalis* spp.), and sedge (*Cyperus* spp.), but also feed on acorns (*Quercus* spp.), and a large variety of seeds and insects (López-Bujanda *et al.*, 2022).

The Montezuma has a patchy distribution with numerous disjunct populations in Arizona, New Mexico, and Texas (Stromberg *et al.*, 2020). This habitat fragmentation may have an additive effect on the loss of genetic variation by genetic drift of isolated populations. For instance, a naturally fragmented habitat has led to reduced levels of genetic variation in the Mexican spotted owl (*Strix occidentalis lucida*) in southeastern Arizona. Montezumas are hunted in Arizona and New Mexico (Stromberg *et al,* 2020; Heffelfinger & Olding 2000), but not in Texas, where populations are restricted to the Trans-Pecos region and the Edwards Plateau (Albers & Gehlbach, 1990). Geographic genetic variation of Texas populations might have been disrupted in the mid 1970s when a series of reintroductions occurred in west and central Texas using Montezumas from Arizona (Wauer, 1973; Stromberg *et al.*, 2020). The success of these reintroductions, and their contribution to the gene pool in those populations, has not been confirmed (Armstrong, 2006).

A population genetic survey is a convenient way to evaluate the effect of isolation, fragmentation, management, and reintroductions on the viability of Montezuma populations. In this regard, the objective of this study was to determine the patterns of genetic variation among Montezuma populations in Arizona, New Mexico and Texas using DNA microsatellite markers. Previous genetic work (Allen, 2003) found that Montezumas from Arizona and Texas were not genetically distinct from one another based on mitochondrial DNA sequences.

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 initially allocated 119 individual samples to four populations: Arizona (AZ), Central New Mexico (CNM, east of the Río Grande), West New Mexico (WNM, west of the Río Grande) and West Texas (WTX). These geographic designations produced an unbalanced sample size, with a relatively low sample size allocated to CNM (n = 12 individuals). Since unbalanced sampling affects the inference of population structure (Meirmans 2019), we re-allocated 14 samples from the two easternmost collection locations of the WNM population to the CNM population. We finally allocated the 119 samples as follows: 32 to AZ, 36 to WNM, 26 to CNM and 25 to WTX (Fig. 1). We used 25 mg of muscle tissue from the right wing 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 were 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 MasterMix manufacturer specifications 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. We used GeneScan™ 350 ROX™ dye Size Standard for sizing DNA fragments. Allele scoring was conducted using program GeneMarker v. 2.6.4 (Hulce *et al*., 2011). We did not run fragment analyses on individual samples more than once and we could not estimate error rates associated with allele scoring. 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. Linkage disequilibrium for each pair of loci across populations was tested using the Fisher's method as implemented by Genepop on the Web (Raymond & Rousset 1995, Rousser 2008).

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 posterior probability of the data given existence of K clusters or groups under Hardy–Weinberg equilibrium and estimates the individuals' posterior membership probability to each of the K clusters. Individuals are then assigned to the cluster that holds the highest probability. 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 Genepop 4.3 (Rousset 2008) to determine if there was a distance isolation pattern among the Montezuma populations. 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

A reduced number of DNA microsatellite loci out of the 20 loci tested were used for genetic analyses. We use locus (Quail) names as in Schable *et al*. (2004) hereafter. Loci Quail 3, Quail 13, Quail 24, and Quail 27 did not amplify for some individuals. For the first three loci, only 1 individual did not amplify in CNM, WNM, and AZ respectively. For Quail 27, three individuals of CNM and two of WNM ~~showed no alleles~~. Quail 41, despite ~~showing~~ amplification of fragments in agarose gels, we could only genotype 21 individuals and this locus was excluded from analyses. ~~The presence of~~ null alleles ~~was~~ detected in Quail 27 and Quail 44, and these were excluded from the analyses of genetic structure (see below). Seven loci were polymorphic in at least one population, while Quail 25 and Quail 44 were monomorphic for all populations (Table 1). We found private alleles for Quail 03, Quail 13, Quail 27, and Quail 31 ~~at~~ populations AZ, CNM and WTX. Quail 27 deviated from ~~the~~ HWE in AZ, CNM and WNM, and had heterozygous deficit. 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. Quail 24 did not meet ~~the~~ HWE at WNM, where many ~~of the~~ individuals ~~presented~~ the same homozygous genotype. There was suggestive evidence of linkage disequilibrium for only pairs Quail 9–Quail

31 (χ^2 = 9.58, d.f. = 4, P = 0.048) and Quail 27–Quail 31 (χ^2 = 16.86, d.f. = 8, P = 0.032) and no evidence of linkage disequilibrium for the other 23 pairs (P > 0.05). Hence, no locus was discarded due to linkage disequilibrium. ~~Finally~~, all samples (n = 119) were analyzed using 9 DNA microsatellite loci.

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

Overall, among-populations genetic differentiation was low (R_{ST} = 0.045). However, pairwise differentiation between distant populations AZ–WTX (R_{ST} = 0.094. P = 0.001), AZ–CNM (R_{ST} = 0.061. P = 0.009), and WTX–WNM had statistical significance (R_{ST} = 0.094, P = 0.001) (Table 2). We found three genetic clusters (K = 3) (Fig. 2), whose membership probabilities ~~showed an evident~~ longitudinal gradient (Fig. 3); study populations~~'~~ geographic positions were not input of the STRUCTURE analysis. Most Montezumas from the AZ ~~population~~ (66%) had a higher probability of membership ~~to~~ one cluster (Arizona cluster hereafter, Fig. 3). The probability of membership ~~to~~ this Arizona cluster ~~within individuals~~ declined towards the east, with only 12% of the WTX individuals assigned to the Arizona cluster. Likewise, most Montezumas from WTX ~~population~~ (48%) had a higher probability of membership ~~to~~ a second cluster (Texas cluster hereafter, Fig. 3). The probability of membership ~~to~~ the Texas cluster within individuals declined towards the west, with only 3% of the AZ individuals assigned to the Texas cluster. However, the probability of membership ~~to~~ the third

cluster (New Mexico cluster hereafter, Fig. 3) showed no geographic gradient (Fig. 3). Populations WNM and WTX had the highest assignment rates to the New Mexico cluster, with 47% and 40% of their individuals, respectively.

Geographical distance and genetic distance (R_{ST}) were strongly correlated across populations ($r^2 = 0.84$) (Fig. 4). Nevertheless, we found only suggestive evidence (Mantel test, $P = 0.08$) of non-independence between geographical distance and genetic distance. According to R_{ST} statistics, AZ and WTX populations had the greatest genetic and geographic distances while CNM and WNM populations had a shorter genetic and geographical distance.

Discussion

Our results and their discussion should be taken with caution due to the relatively low number of loci analyzed (9 loci). Low heterozygosity ($\overline{H}_O = 0.22 \pm 0.02$), few alleles per locus ($A = 2.41 \pm 0.27$) and a high proportion of fixed loci (3 out of 9 loci) demonstrate low genetic variability of the Montezuma in the southwestern United States. For instance, DeYoung *et al*. (2012) reported an average observed heterozygosity of $H_o = 0.58$ in seven DNA microsatellite loci for bobwhite (*Colinus virginianus*) populations in southern Texas. Orange *et al.* (2014) reported a H_o from 0.250 – 0.928 and an average $A = 7$ in 23 DNA microsatellite loci for scaled quail (*Callipepla squamata*) populations in Arizona, Colorado, Oklahoma, and Texas. Similarly, Mathur *et al* (2019) reported low levels of genetic variability in the Montezuma populations in Arizona, New Mexico and West Texas using genome-wide single nucleotide polymorphism, with $H_o = 0.32 \pm 0.17$. Low genetic variability observed on Montezuma populations may be caused by drift on small and isolated populations inhabiting oak-pine-juniper savanna islands separated by vast desert grasslands. In this regard, Lesica & Allendorf (1995) state that low migration rates between the peripheral populations and those closer to the center of the species' geographic

range could promote gene drift, ~~hence,~~ fixed alleles and reduction of genetic variability are more likely to occur. For instance, the Mexican spotted owl ~~showed~~ reduced levels of genetic (mtDNA) variation in the Madrean sky islands of southeastern Arizona (co-inhabited by Montezumas) compared to the remaining owl populations in Arizona, New Mexico, Utah, and Colorado (Barrowclough *et al*. 2006).

Slight population structure was shown through the low R_{ST}. ~~Still,~~ pairwise R_{ST} values indicate a low to moderate differentiation in some study populations. For instance, AZ is different from CNM (R_{ST} = 0.061; P = 0.011) and WTX (R_{ST} = 0.094; P = 0.001). In addition, AZ is not differentiated from WNM, suggesting an extant corridor for Montezumas between these two study populations. Montezumas occur through the forested Mogollon Rim, which may connect WNM to central Arizona and to mountain ranges to southern Arizona (AZ) as both E-bird sightings (Sullivan *et al*., 2009) and GBIF (GBIF.org, 2023) records suggest (Fig. 1). The Montezuma's ability to disperse this distance may not be as limited as presumed. A radiotelemetry study conducted in Texas found that Montezumas ~~can~~ move up to 15 km (Greene *et al.*, 2020). Furthermore, the lowest genetic differentiation occurred between neighboring WNM and CNM (R_{ST} = 0.014; P = 0.063). Although some CNM individuals appear to be geographically isolated from the rest of the population (for example, individuals collected in the Lincoln National Forest), Montezumas can occur in areas with riparian vegetation near oak forest (Stromberg *et al*, 2020), hence, they may be using riparian corridors to transit between the WNM and CNM populations. Sightings on the Rio Grande (Sullivan *et al*., 2009) also suggest connectivity between WNM and CNM through this river.

Despite a low genetic differentiation among our study populations, patterns of genetic variation from all our analyses suggests isolation by distance, which arises from limited

geneflow between distant populations (Wright, 1943). R_{ST} values between distant populations 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 previous mtDNA survey by Allen (2003) found no genetic differentiation between Montezuma populations in Arizona and Texas. Differentiation between neighboring WTX–CNM populations (R_{ST} = 0.033) was suggestively ~~significant~~ (P = 0.096), ~~suggesting~~ gene flow between these populations >200 km apart. ~~Again, Greene *et al.* (2020) reported movements of up to 15 km for Montezumas in this region of Texas, 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 study populations. However, the current existence of a large extension of unsuitable arid land between the two mountainous areas and the ~~highest non-significant~~ R_{ST} may suggest recent divergence. Furthermore, our Bayesian ~~assignment~~ analysis suggested three clusters, whose assignment probabilities among Montezumas followed a longitudinal gradient (Fig. 3), although mixing between clusters was evident. However, a Bayesian assignment by Mathur *et al*. (2019) did not detect separation between Arizona and New Mexico using genomic-wide single nucleotide polymorphism. Nevertheless, Mathur *et al.* (2019) also found lower differentiation between the populations of AZ–WNM and WNM–CNM. Thus, these populations may represent a single cluster, while the WTX population would be isolated from the rest. ~~In this regard,~~ approximately 40% of WTX Montezumas were assigned to the NM Cluster, suggesting recent admixture between clusters. Results from this work support the idea, proposed by Mathur *et al.* (2023), that the Montezuma 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. Alternatively, our results may also suggest gene flow has recently stopped. Connectivity between Montezuma populations in the American southwest and northern Mexico may have been more widespread before the late 19th century when extensive ranching began reducing and depleting the grass cover (Humprey, 1958) needed by Montezumas to disperse.

The Mantel test also showed suggestive evidence of isolation by distance in Montezuma populations. Changes in temperature and precipitation patterns apparently expanded in altitude and latitude boundaries of deserts surrounding the Montezuma habitat in the southwestern United States (Archer & Predick, 2008; Seager *et al*., 2007; Williams *et al*., 2010). In this context, constant fragmentation, and predicted reduction of the extent of currently suitable habitats for the Montezuma (Tanner *et al*., 2017) may reduce corridors between populations, causing complete separation of populations and increasing the risk of survival of the species in the medium and long term.

Given the low genetic structure found in this study and the resolution offered by DNA microsatellites, we could not detect the effects of Montezuma reintroductions from Arizona to Texas on genetic structure. Still, there is no evidence that the reintroductions were successful and that any genetic structure was disrupted. However, we found a significant genetic difference between AZ and WTX. In addition, Mathur *et al*. (2023) also found that extant populations in Arizona and Texas are genetically distinct from one another, having diverged approximately 17,000 years ago.

The presence of isolated populations in mountain patches of habitat or “sky islands” is frequent along all edges of the species’ geographic distribution, which may be embedded in arid, subtropical, or tropical vegetation. The existence of these isolated populations poses a

conservation challenge for managers. These isolated populations have most likely differentiated, as those of numerous vertebrates inhabiting sky islands systems (Barrowclough 2006, Browne and Ferree 2007, Hartley *et al*. 2023; Love *et al.*, 2023). The degree to which isolated Montezuma populations have differentiated deserves investigation. Montezumas in sky islands may have a similar degree of differentiation that led to the special management status of the Mount Graham red squirrel (*Tamiascirus fremonti grahamensis*) in Arizona (U.S. Fish and Wildlife Service, 1987). Furthermore, isolation in sky islands can also lead to local adaptation, which may also promote persistence of a species. Therefore, a genomic survey through the Montezuma's geographic distribution is research priority. Genome sequencing has been undertaken for the species (Mathur *et al*., 2019; Mathur & DeWoody, 2021; Mathur *et al.*, 2023), but with minimal representation of Mexican and sky island populations. Genomic surveys will unveil genetic structure patterns relevant for conservation. For instance, translocations from one island population to another by federal and state game management agencies in the United States may have irreparably disrupted local genetics in such isolated populations. Like the Mount Graham's red squirrel, genomic studies of Montezuma populations in Texas, southeastern Arizona, and southwestern New Mexico sky islands might reveal that they may warranted special management status.

Conclusions

The subtle genetic structure and low levels of genetic variation detected in our study is valuable for the future management of this charismatic game bird. This subtle genetic structure consists in clusters Arizona, New Mexico, and 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. Montezuma populations in the southwestern United States may not be fully isolated, despite habitat loss and fragmentation in this region as some corridors may open or close periodically depending on annual precipitation and its effect on herbaceous vegetation cover.

Acknowledgements

We thank Robert Perez of the Texas Parks and Wildlife Department for his logistical support that made the collection of Montezuma specimens in Texas possible. We also thank the volunteer hunters who collected or assisted in collecting specimens including Randy Gray, Steve Hopkins, Dennis Kavanagh, Zack May, Bill Miller, Mike Sullins, Ray Trejo, Carlos Zuniga, 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.

Allen, T. H. 2003. Mitochondrial genetic variation among populations of Montezuma Quail (*Cyrtonyx montezumae*) in the southwestern United States. M.S. thesis. Sul Ross State University. Alpine, Texas, United States of America.

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.

320 Arif, I.A. & H.A. Khan. 2009. Molecular markers for biodiversity analysis of wildlife animals: a
321 brief review. *Anim. Biodiv. and Conserv*. 32:9-17.
322 Armstrong, K. 2006. Texas quails: ecology and management. Texas A&M University Press.
323 Texas, United States of America.
324 Barrowclough, G. F., J. G. Groth, L. A. Mertz, & R. J. Gutiérrez. 2006. Genetic structure of
325 Mexican spotted owl (*Strix occidentalis lucida*) populations in a fragmented landscape.
326 *The Auk* 123:1090–1102.
327 Bishop, R. A. 1964. The Mearns Quail (*Cyrtonyx montezumae mearnsi*) in southern Arizona.
328 M.S. thesis, University of Arizona, Tucson, United States of America.
329 BirdLife International. 2016. *Cyrtonyx montezumae*. The IUCN Red List of Threatened Species
330 2016:e.T22728212A94975065.
331 http://dx.doi.org/10.2305/IUCN.UK.20163.RLTS.T22728212A94975065.en. Downloaded on 11
332 December 2017.
333 Brown, D. E. 1979. Factors influencing reproductive success and population densities in
334 Montezuma Quail. *J. Wildlife Manag*. 43:522–526.
335 Browne R. A., & P. M. Ferree. 2007. Genetic Structure of Southern Appalachian "Sky Island"
336 populations of the Southern Red-Backed Vole (*Myodes gapperi*). J. *Mamm.* 88:759–768.
337 Chavarria, P. M., N. J. Silvy, R.R. Lopez, D. S. Davis, & A. Montoya. 2017. Ranges and
338 movements of Montezuma quail in southeast Arizona. *Proceedings of the National Quail*
339 *Symposium* 8:94.
340 DeYoung, R. W., E. M. Wehland, D. L. Williford, A. Zamorano, J. P. Sands, L. A. Brennan, F.
341 Herna´ndez, S. J. DeMaso, & R. M. Perez. 2012. Genetic structure and diversity in South

342 Texas bobwhites: implications for conservation. *National Quail Symposium Proceedings*
343 7:252–253.
344 Earl, D. A. 2012. Structure Harvester: a website and program for visualizing Structure output and
345 implementing the Evanno method. *Conserv. Genet. Resour.* 4:359-361.
346 Evanno, G., S. Regnaut, & J. Goudet. 2005. Detecting the number of clusters of individuals
347 using the software Structure: a simulation study. *Mol. Ecol.* 14:2611-2620.
348 Excoffier, L. & H.E. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform
349 population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 10:564-567.
350 Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. *Conserv.*
351 *Biol.* 10:1500-1508.
352 GBIF.org. 2023. GBIF Occurrence Download https://doi.org/10.15468/dl.53a6gj. Downloaded
353 on 28 July 2023.
354 Greene, C. D., Harveson, L. A., Cross, J. G., & Luna, R. S. 2020. Spatial ecology and habitat
355 utilization of Montezuma quail in the Davis mountains of Texas. *Texas J. Sci.* 72(1):
356 Article-4.
357 Harris, R. B., W. A. Wall, & F. W. Allendorf. 2002. Genetic consequences of hunting: what do
358 we know and what should we do? *Wildlife Soc. B.* 30:634–643.
359 Hartley, R., N. Clemann, Z. Atkins, B. C. Scheele, D. B. Lindenmayer, and M. D. Amor. 2023.
360 Isolated on sky islands: genetic diversity and population structure of an endangered
361 mountain lizard. *Conserv. Gen.* 24:219–233.
362 Heffelfinger, J. R. & R. J. Olding. 2000. Montezuma quail management in Arizona. *National*
363 *Quail Symposium Proceedings* 4:45.

364 Hulce, D., X. Li, T. Snyder-Leiby, & C. J. Liu. 2011. GeneMarker® genotyping software: Tools
365 to increase the statistical power of DNA fragment analysis. *JBT* 22(Suppl), S35.
366 Humprey, R. R. 1958. The desert grassland, a history of vegetational change and an analysis of
367 causes. *Bot. Rev.* 24:193–252.
368 Instituto Nacional de Estadística y Geografía. 2022. Marco Geoestadístico. Available at
369 https://www.inegi.org.mx/temas/mg/#mapas (accessed August 12, 2023).
370 Lesica, P. & F.W. Allendorf. 1995. When are peripheral populations valuable for
371 conservation? *Conserv. Biol.* 9:753–760.
372 Love, S. J., J. A. Schweitzer, S. A. Woolbright, & J. K. Bailey. 2023. Sky Islands Are a Global
373 Tool for Predicting the Ecological and Evolutionary Consequences of Climate Change.
374 *Annu. Rev. Ecol. Evol. S.* 54:1
375 Mathur, S., J. M. Tomeček, A. Heniff, R. Luna, & J. A. DeWoody. 2019. Evidence of genetic
376 erosion in a peripheral population of a North American game bird: the Montezuma quail
377 (*Cyrtonyx montezumae*). *Conserv. Gen.* 20:1–13.
378 Mathur, S. & J. A. DeWoody, 2021. Genetic load has potential in large populations but is
379 realized in small inbred populations. *Evol Appl*. 14:1540–1557.
380 Mathur, S., J. M. Tomeček, L. A. Tarango-Arámbula, R. M. Perez, & J. A. DeWoody. 2023. An
381 evolutionary perspective on genetic load in small, isolated populations as informed by
382 whole genome resequencing and forward-time simulations. *Evolution* 77:690–704.
383 Meirsman, P. G. 2019. Subsampling reveals that unbalanced sampling affects STRUCTURE
384 results in a multi-species dataset. *Heredity* 122:276–287.
385 National Weather Service. 2023. U.S. States and Territories. Available at
386 https://www.weather.gov/gis/USStates (accessed August 12, 2023).

387 Orange, J. P., C. A. Davis., R.A. Van Den Bussche., S. D. Fuhlendorf., D. R. Elmore., E. T.
388 Thacker, & M. E. Judkins. 2014. Development and characterization of 23 microsatellite
389 loci for scaled quail (*Callipepla squamata*). *Conserv. Genet. Resour.* 6:929–931.
390 Pritchard, J. K., M. Stephens, & P. Donnelly. 2000. Inference of population structure using
391 multilocus genotype data. *Genetics* 155:945–959.
392 Raymond M. & Rousset F, 1995. GENEPOP (version 1.2): population genetics software for
393 exact tests and ecumenicism. *J. Heredity* 86:248-249
394 Reed, D. H. & R. Frankham. 2003. Correlation between fitness and genetic diversity. *Conserv.*
395 *Biol.* 17:230–237.
396 Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for
397 Windows and Linux. *Mol. Ecol. Resources* 8:103–106
398 Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for
399 Windows and Linux. *Mol. Ecol. Resources* 8:103–106.
400 Schable, N. A., B. C. Faircloth, W. E. Palmer, J. P. Carroll, L. W. Burger, L. A. Brennan, C.
401 Hagen, & T. Glenn. 2004. Tetranucleotide and dinucleotide microsatellite loci from the
402 northern bobwhite (*Colinus virginianus*). *Mol. Ecol. Notes* 4:415–419.
403 Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H.P. Huang, N. Harnik, A. Leetma,
404 N. C. Lau, C. Li, J. Velez, & N. Naik. 2007. Model projections of an imminent transition to
405 a more arid climate in southwestern North America. *Science* 316:1181–1184.
406 Smouse, R. P. P. & R. Peakall. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic
407 software for teaching and research—an update. *Bioinformatics* 28:2537–2539.
408 Stromberg, M. R. 1990. Habitat, movements and roost characteristics of Montezuma quail in
409 southeastern Arizona. *Condor* 92:229–236.

Stromberg, M. R., A. B. Montoya, & D. Holdermann. 2020. Montezuma Quail (*Cyrtonyx montezumae*), version 1.0. In Birds of the World (P. G. Rodewald, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.monqua.01

Sullivan, B. L., C. L. Wood, M. J. Iliff, R. E. Bonney, D. Fink, & S. Kelling. 2009. eBird: a citizen-based bird observation network in the biological sciences. *Biol. Conserv*. 142: 2282–2292.

Tanner, E. P., M. Papeş, M., R. D. Elmore, S. D. Fuhlendorf, & C. A. Davis. 2017. Incorporating abundance information and guiding variable selection for climate-based ensemble forecasting of species' distributional shifts. *PLoS One* 12: e0184316.

U.S. Fish and Wildlife Service. 1987. Endangered and threatened wildlife and plants; determination of endangered status for the Mount Graham red squirrel. Federal Register 52:20, 994–999

Van Oosterhout, C., W. F. Hutchinson., D. P. Wills. & P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Resour.* 4(3): 535–538.

Wauer, R. H. 1973. Birds of Big Bend National Park and vicinity. University of Texas Press. Texas, United States of America.

Waters, J. M., B. C. Emerson, P. Arribas, & G. A. McCulloch. 2020 Dispersal reduction: causes, genomic mechanisms, and evolutionary consequences. *Trends Ecol. Evol*. 35: 512-522

Williams, A.P., C. D. Allen, C. I. Millar, T. W. Swetnam, J. Michaelsen, C. J. Still, & S. W. Leavitt. 2010. Forest responses to increasing aridity and warmth in the southwestern United States. *P. Natl. Acad. Sci. USA* 107:21289-21294.

Wright, S. 1943. Isolation by distance. *Genetics* 28:114–138.

Figure 1

Sampling locations of 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 (AZ), West New Mexico (WNM), Central New Mexico (CNM), and West Texas (WTX), respectively. Gray dots are records from e-bird (Sullivan *et al.*, 2009) and GBIF (GBIF.org, 2023). State division for the United States drawn from data by National Weather Service (2023). State division for Mexico drawn from data by Instituto Nacional de Geografía y Estadística (2022).

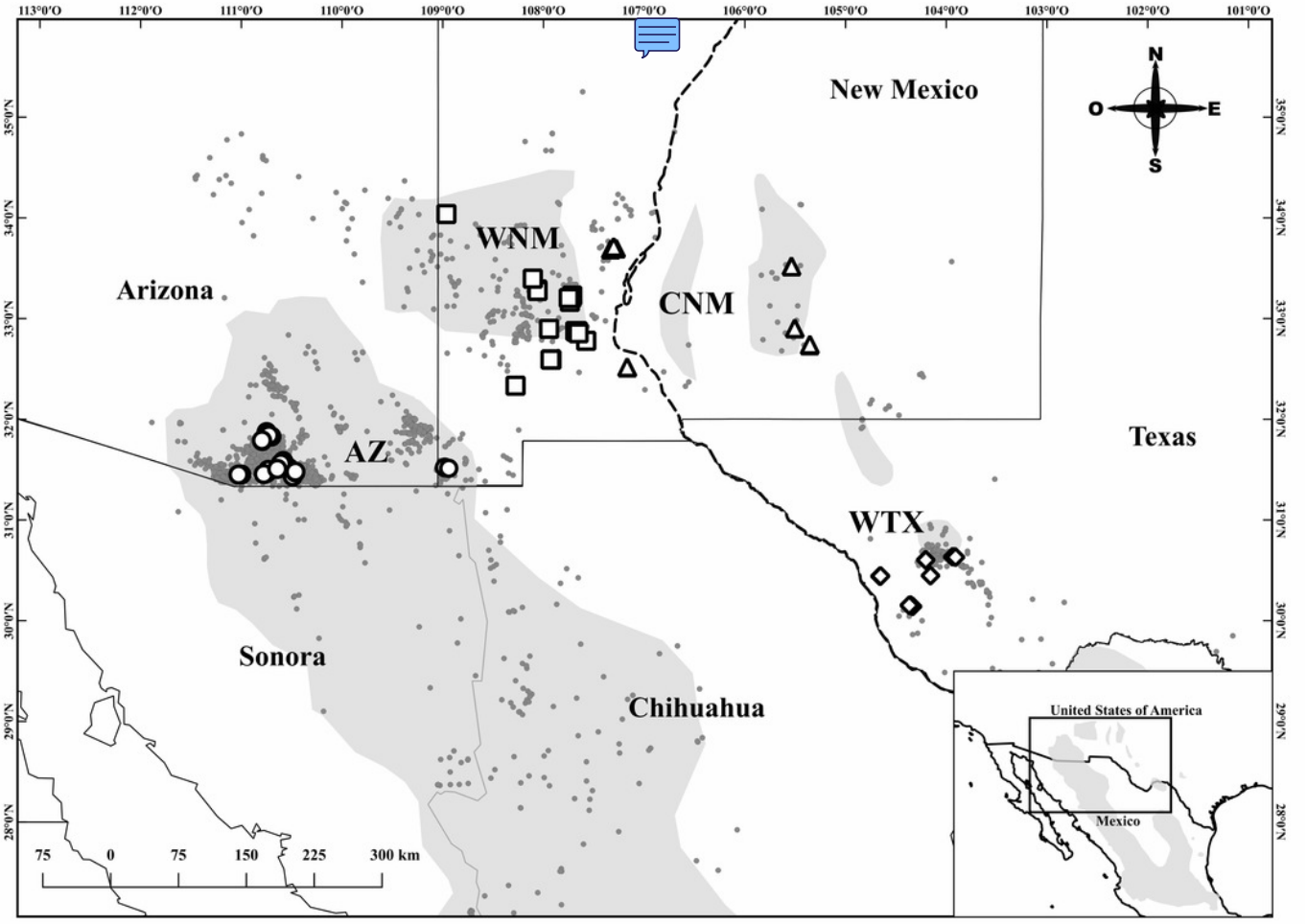

Figure 2

Log-likelihood values *vs*. number of groups from the Bayesian method obtained in program STRUCTURE (Pritchard *et al*., 2000) (*ΔK*) for DNA microsatellite data of Montezuma quail from Arizona, New Mexico, and Texas.

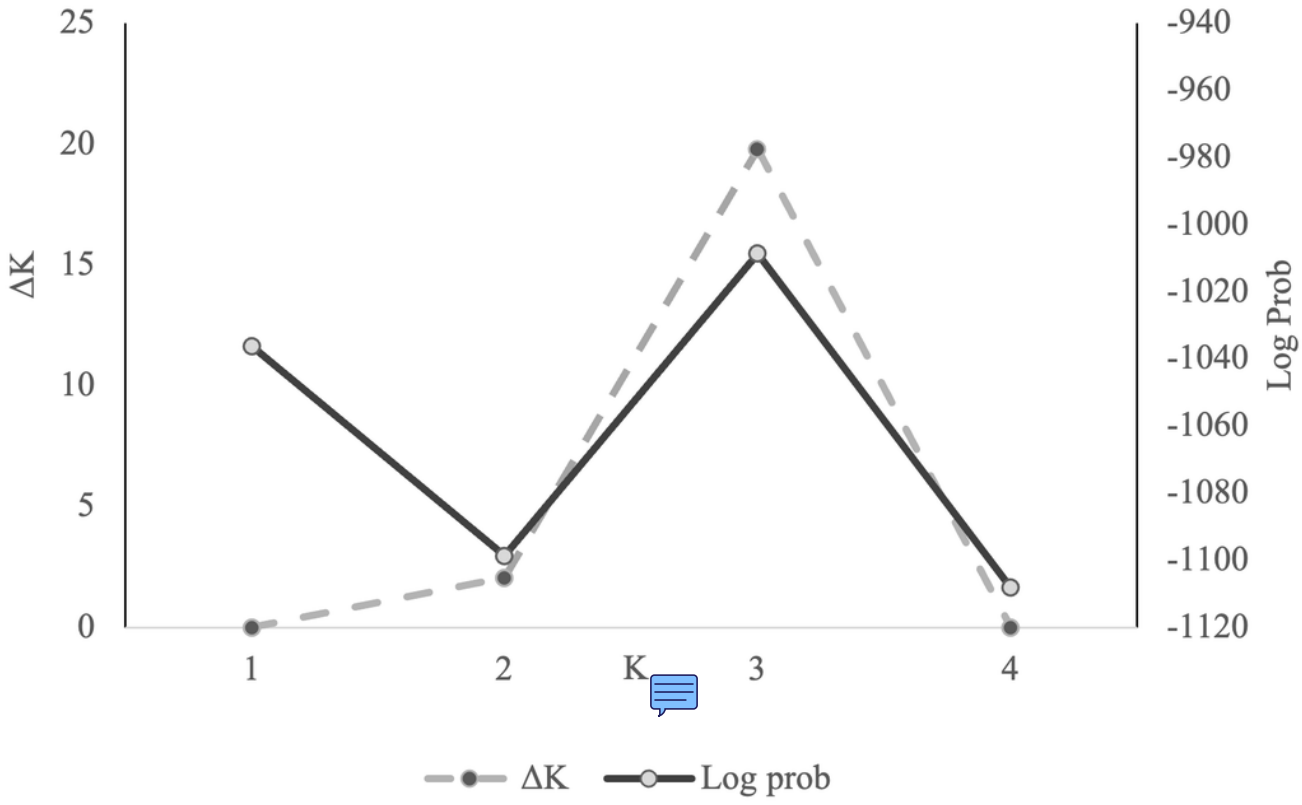

Figure 3

Geographic variation among Montezuma quail populations in the posterior membership probability to each of the clusters inferred by program STRUCTURE (Pritchard *et al*. 2000).

Each stacked bar represents an individual's ancestry estimates of each genetic cluster (Arizona, New Mexico, and Texas). Colors purple, blue, and yellow correspond to individuals' probability of membership to clusters Arizona, New Mexico, and Texas, respectively.

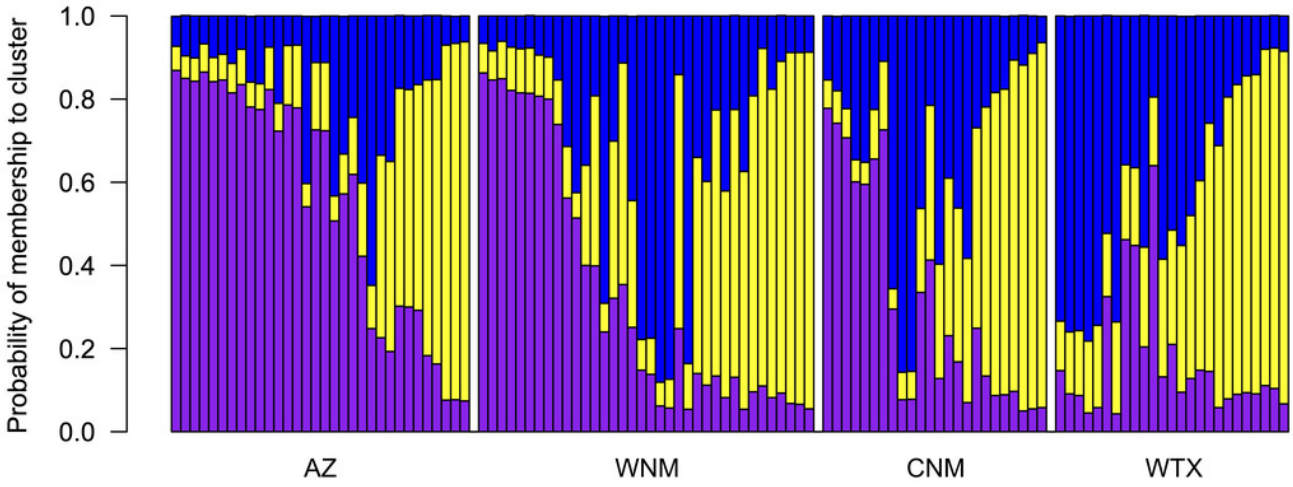

Figure 4

Correlation between geographical distance and the R_{ST} statistic values for each pair of populations (Mantel test, $r^2 = 0.84$, $P = 0.08$).

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.

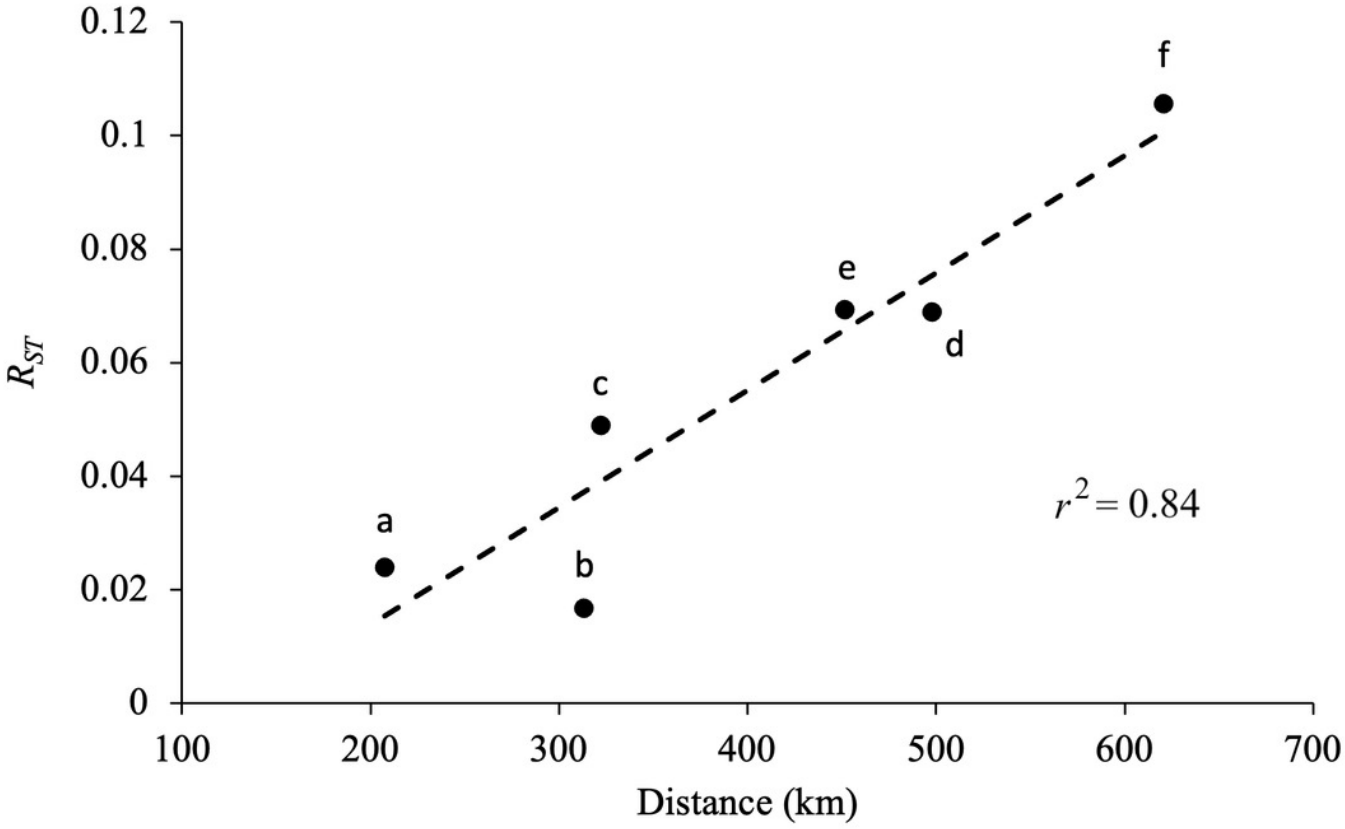

Table 1(on next page)

Genetic variability estimators for nine microsatellite loci of Montezuma quail populations in 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

Population	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