GENETIC STRUCTURING OF COUES WHITE-TAILED DEER IN THE SOUTHWESTERN UNITED STATES

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ABSTRACT

GENETIC STRUCTURING OF COUES WHITE-TAILED DEER

IN THE SOUTHWESTERN UNITED STATES

Roy G. Lopez

The manuscripts in this thesis examine different aspects of white-tailed deer. In the first manuscript I used microsatellite DNA markers in the form of multilocus genotype data and microsatellite allele frequencies to examine spatial patterns of genetic relatedness for Coues white-tailed deer (*Odocoileus virginianus couesi*) in Arizona and New Mexico in naturally fragmented habitats (sky islands) and in relatively continuous habitats (Mogollon Rim). I determined genotype, based on 12 microsatellite markers for 358 Coues white-tailed deer from these two regions. Both population and individual statistics indicate genetic differentiation between the "mainland" (Mogollon Rim) subpopulation and a sky island subpopulation.

In the second manuscript I explore the extent of hybridization between Coues white-tailed deer and desert mule deer *(O. hemionus eremicus).* Both species are sympatric in many areas of the southwestern United States. Hybrid Coues white-tailed deer and desert mule deer have been documented in controlled experiments and in rare instances in the wild. I used microsatellite DNA markers to investigate the extent of natural hybridization between Coues white-tailed deer and desert mule deer. My objective was to determine how commonly this occurs in wild populations of Coues

white-tailed deer in the southwestern United States. My results suggest about 2% of wild free ranging Coues white-tailed deer in Arizona and New Mexico are hybrids, including some animals that are probably backcrosses to each parental species.

In the process of collecting tissue samples, naturally cast antlers became a potentially important source of tissue from white-tailed and mule deer. The extent of natural weathering on cast antlers resulting in degradation of DNA was unknown. I collected and tested cast antler in various stages of natural decomposition to determine the amount of weathering cast antlers could endure and still yield usable DNA. Based on physical characteristics of cast antlers, they were placed into seven weathering classes ranging from freshly cast and not subjected to excessive heat and moisture while lying in the field to antlers estimated at having been exposed to 8-10 years of weathering in the field. Most cast antlers yielded DNA. Precautions in extracting tissue from cast antlers were identified to avoid scorching tissue samples.

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I thank my committee, Paul Beier, Laura DeWald, Al Medina and Phil Service. I would like to especially thank Paul Beier for his support and guidance.

Fresh tissue for this research was provided by hundreds of Coues white-tailed deer hunters, Arizona Game and Fish Department wildlife managers, taxidermists, Sheridan Stone of Fort Huachuca and The Animas Foundation. Cast antler tissue was provided by numerous shed antler collectors, especially Chris Dunn, Dan King and Jeff Rivera. Without these people's help, I could not have conducted this research.

I would like to thank members of the Beier lab group for providing comments and feedback on this research and on practice presentations. I would like to especially thank Brad McRae who took the time to answer the myriad of questions that I had.

Jim Heffelfinger, Arizona Game and Fish Department Regional Big Game Specialist, was instrumental in providing Hybrid tissue samples. Yar Patryszyn, University of Arizona Mammal Collection Manager, allowed me to collect bone shaving from the two hybrid Coues X mule deer specimens in their collection. Jeff Jenness produced the general range and density map in chapters 1 and 2, adapting it from R. Ockenfels distribution and density map of Coues white-tailed deer in Arizona and J. Heffelfinger and D. Semmens general range map of Coues white-tailed deer in Arizona. Laurie Porth, statistician at RMRS, helped run the "zt" Mantel test. Bonnie Yates helped

identify the bacterial growth found on cast antlers exposed to the elements for many years.

Finally, I thank my family. Thank you, Gabriel and Andrea, for being full of questions and covering my office walls with beautiful art work. I especially thank my wife Sharon for her unending moral support and feedback. Also, I thank her for anticipating that it might not be a good idea to send oddly-shaped tissue collection kits to hunters, who were not expecting them, after the anthrax mail attacks in 2001, because this might lead to law enforcement agencies calling me to find out what I was doing, which they did.

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PREFACE

The chapters in this thesis are each written as stand-alone journal articles, with each chapter written in the required format for the prospective journal. In Chapter 1, I use molecular genetic techniques to examine the genetic pattern of Coues white-tailed deer (*Odocoileus virginianus couesi*) in the southwestern United States in two landscapes in New Mexico and Arizona - the naturally fragmented sky islands and the continuous landscape along and below the Mogollon Rim. These landscapes provide an ideal system for studying the influence of habitat pattern on genetic patterns, as Coues white-tailed deer are associated with woodland habitats. Chapter 1 will be submitted as a manuscript to Molecular Ecology, Journal of Wildlife Management, or Animal Ecology. Chapter 2 explores the extent of hybridization between Coues white-tailed deer and mule deer in wild populations in the southwestern United States and will be submitted to The Southwestern Naturalist. Chapter 3 describes the use of weathered antlers as a source of DNA for population genetic analysis. It will be submitted as a manuscript to Western North American Naturalist.

Identifying subpopulations of Coues white-tailed deer the southwestern United States using multilocus genotype data.

Abstract: I used multilocus genotype data and microsatellite allele frequencies to examine spatial patterns of genetic relatedness for Coues white-tailed deer (*Odocoileus virginianus couesi*) in Arizona and New Mexico in naturally fragmented habitats (sky islands) and in relatively continuous habitats (Mogollon Rim). Because these deer are associated with oak woodland and oak-pine woodlands, relatively high levels of gene flow are expected along the Mogollon Rim where no habitat barriers to movement exist. Conversely, gene flow should be restricted in areas where habitat discontinuities exist, as among the sky island region of SE Arizona and SW New Mexico, which is characterized by basin and range topography with desert grasslands separating forested mountains. I determined genotype, based on 12 microsatellite markers for 358 Coues white-tailed deer from the sky islands of southeastern Arizona and southwest New Mexico and from along and below the Mogollon Rim from southwest of Flagstaff Arizona to the Black Range of New Mexico. As expected, the sky islands showed a stronger pattern of isolation by distance than did deer populations along the Mogollon Rim. Both population and individual statistics indicated genetic differentiation between a "mainland" (Mogollon Rim) subpopulation and a sky island subpopulation. I also found evidence for unexpectedly low gene flow between some pairs of neighboring sampling units, which may reflect a combination of habitat and anthropogenic barriers.

INTRODUCTION

The study of genetic structuring among natural populations occupying patchy landscapes has wide application to questions of biogeography, metapopulation dynamics, spatial ecology and conservation of endangered species. Closely spaced patches (separated by distances within the animal's dispersal ability) should show smaller genetic distances at close distances than at far distances **(**Wright 1943**).** As interpatch isolation increases beyond the organism's dispersal range, genetic divergence among neighboring populations is usually correlated with degree of isolation.

Vicariance processes, associated with discontinuities in woodland and forest vegetation after the Pleistocene, have been a major factor structuring patterns of differentiation among allopatric mammal populations in the Great Basin of North America (Brown 1971). In the sky island region of the southwestern United States (where high elevation woodlands occur within a matrix of desert vegetation), many species of woodland- associated mammals are expected to exist as allopatric or vicariant populations in contrast to populations distributed throughout the Mogollon highlands where woodlands are continuous, although density may vary greatly depending on woodland quality. Vicariant genetic divergence associated with degree of isolation has been observed in several non volant small mammals of the southwestern United States, including least chipmunk *Eutamius minimus* (Sullivan 1985), pocket gophers *Thomomys umbrinus* (Hafner et al. 1987), and Mexican wood rats *Neotoma mexicana* (Sullivan 1994). McRae's et al. (2005) research on pumas (*Felis concolor*) in the southwestern United States found greater genetic differentiation in the fragmented habitat of the sky island region compared to pumas found in more continuous habitat. Pumas in the

southwestern United States use a variety of habitats and have exhibited the ability to disperse great distances. If vicariant processes dominate in a fragmented landscape such as in the sky island region and isolation by distance dominates in a continuous landscape, white-tailed deer in the fragmented landscape might be expected to exhibit more genetic substructure and have higher genetic distance between populations at a given Euclidian distance than in a continuous landscape.

Coues white-tailed deer (*Odocoileus virginianus couesi*) are strongly associated with woodland habitats in the southwestern United States, have a more limited dispersal ability compared to pumas and thus serve as an ideal species to contrast with smaller mammals.

Arizona Game and Fish Department has designated Game Management Units (GMU's) that determine where hunters are allowed to hunt game species such as white-tailed, and sets harvest goals for white-tailed deer in each GMU. Although GMU boundaries are set based on manager's opinion of population subdivisions of big game species, they may not correspond to biological boundaries for these species. .

In this paper, I describe genetic structuring of Coues white-tailed deer in two different landscapes of Arizona and southwestern New Mexico: naturally fragmented habitats (sky islands) and relatively continuous habitats (Mogollon Rim). These data were used to quantify the relationship between genetic divergence and geographic distance in each landscape. I conducted analyses based on individual samples, as well as geographic clusters of samples. My primary goal was to examine empirical support for the relative importance of vicariance distribution versus isolation by distance for this large mammal. In addition, I wished to (a) describe genetic patterns as a baseline to

detect future changes, and (b) test whether current GMU's correspond to biologically meaningful boundaries for this species.

STUDY AREA

The sky island region of southeastern Arizona and southwestern New Mexico is characterized by basin and range topography, with mountains 1000 -2000 meters above the valley floor dominated by Madrean pine-oak woodland separated by desert grassland valleys. I use the term "sky island" (Heald 1967) to refer to individual mountain ranges in this region. The term Mainland refer to the area of continuous habitat above, along and below the Mogollon rim, an escarpment rising to 2,100 m defining the southwestern edge of the Colorado Plateau, and the Mexican Sierra Madre Occidental where extensive ponderosa pine (*Pinus ponderosa*) forests are found both on the slopes of the rim and on the plateau above. This area extends approximately 800 km from northern Yavapai County southwest of Williams Arizona eastward to the Black Range of the Gila National Forest New Mexico.

About 10,000 years ago, basins (550-1,500 m elevation) between the sky islands were characterized by piñon -juniper-oak woodlands. These shifted to desert grassland and Sonoran desert scrubland about 4,000 years ago (Van Devender et al. 1987, Finley 1990, Van Devender 1990, Turner and Brown 1994). These piñon-juniper-oak woodlands were probably inhabited by Coues white-tailed deer, as these same biotic communities currently are the core of white-tailed deer habitat. The isolated sky islands and mountain range along the Mogollon Rim where Coues white-tailed deer now have the highest densities (Figure 1) were dominated by montane conifer forests and sub-alpine woodland (Betancourt et al. 1990) and probably not suitable for Coues white-

tailed deer. As the climate changed and vegetational communities shifted upward altitudinally white-tailed deer presumably shifted their range accordingly.

A single subspecies of white-tailed deer occurs in the study area, Coues white-tailed deer (Mearns, E. A. 1907, Bailey 1931, Hoffmeister 1962, Raught 1967, Findley 1975, Baker 1984). Coues white-tailed deer inhabit most southeastern and central mountain ranges below the Mogollon Rim, primarily in mixed oak woodlands and higher elevation semi-desert grasslands (Hoffmeister 1962, Knipe 1977, Evans 1984). Coues white-tailed deer also occur locally in high desert scrublands, along riparian corridors, and in pine forests along the Mogollon Rim (McCullough 1967, Hoffmeister 1986). Habitats occupied by white-tailed deer characteristically receive a yearly average of 32cm of precipitation, of which more than 15cm can be expected to fall during the summer months. In much of the arid southwest, white-tailed deer usually occur as isolated populations on mountains, high mesas and along ridge lines above an altitude of 1,500 m (Brown 1984).

METHODS

Sample collection

I collected muscle, hide and antler tissue samples from 358 Coues white-tailed deer. Muscle and hide samples were from legally hunted deer collected from hunters between November 2001 and December 2003. Seventy-six samples were from naturally cast antlers that were collected in the field between May 1993 and May 2003. Twenty six desert mule deer *(O. hemionus eremicu)* tissue samples were collected and submitted (not labeled as such) to the genetics laboratory as a quality control check.

I used cast antlers as a source of tissue in areas where hunter harvested Coues deer was low. Locations of hunter harvested and cast antlers from Coues white-tailed deer were mapped using Arc View GIS 3.3 (ESRI, Inc.).

Genetic Analysis

Protein electrophoresis and mitochondrial DNA have been used to detect genetic variation at biparentally inherited loci in North American ungulates (Manlove et al. 1976, Baccus et al. 1983, Cronin et al. 1991). With the exception of white-tailed deer, previous protein electrophoresis studies have revealed low levels of genetic heterozygosity in ungulates (Honeycutt 2000). Thus low heterozygosity limits the utility of this genetic marker. Therefore I studied the more polymorphic microsatellite markers. Microsatellites are tandemly repeated sequences composed of repeat units (2-5 bases) flanked by unique sequences that are dispersed throughout the mammalian genome (Tautz and Renz 1984, Tautz 1989, and Weber and May 1989). Microsatellite markers are common in eukaryotic genomes and can be amplified by the polymerase chain reaction, which allows the use of minute or degraded samples (Hughes and Queller 1993). The high mutation rates and high levels of polymorphism associated with microsatellites make these markers ideal for studying patterns of gene flow, responses of recent population subdivision as a consequence of habitat fragmentation, and population bottlenecks (Honeycutt 2000) and have been validated for genetic exclusion studies in white-tailed deer (DeYoung et al. 2003).

Microsatellite DNA loci have been characterized for several species of ungulates including white-tailed deer (DeWoody et al.1995, Anderson et al. 2003) caribou (*Rangifer tarandus*) (Wilson et al.1997) and mule deer (Jones et al. 2000). The unique

primer sites flanking many DNA microsatellite markers are conserved across taxa allowing microsatellite loci isolated from one species to be used in related species (Engel et al. 1996, Wilson et al 1997).

Wildlife Genetics International, (Nelson, British Columbia Canada) scored these samples at 12 microsatellite genetic markers, nine dinucleotide repeats and three tetranucleotide repeats (Table 1). Microsatellite analysis used Applied Biosystems (ABI) four color detection system on a 373A automated sequencer and genotypes were determined using Genotyper software (ABI). The 12 loci used were PCR amplified in eight reactions. Mixing reactions together after amplification allowed all loci from a single individual to be run in two gel lanes. Thermal cycling was performed using a Perkin Elmer 9600. Annealing temperature was 54 C. Primers and sequence in Table 2. Estimating the number of genetically distinct clusters from individual genotypes

I used The Excel Microsatellite Toolkit (Park S.D.E. 2001) to check for identical samples. I used a Bayesian clustering method (STRUCTURE 2.3.1, Prichard et al.2000) to infer number of populations and assign individuals to populations based only on multilocus geneotype data. For each postulated number of populations, K, the program calculated the log likelihood probability of the data, Ln(P|D), and the probability of individual membership in each cluster using a Markov chain Monte Carlo (MCMC) method. I varied K from 1-10, using 500,000 MCMC cycles for burn-in (the number of simulation runs before collecting data to minimize the effect of the starting configuration, Prichard and Wen 2003) and 500,000 cycles after burn-in. The value of K with the highest probability of the data (Ln(P/D) indicates the most likely number of genetically distinct clusters.

Comparing the importance of vicariance and isolation by distance

After the previous analysis suggested that deer in the Mogollon Rim Mainland were genetically distinct from deer in the Sky Islands (see *Results*), I conducted analyses to determine whether vicariant processes were relatively important in the Sky Islands compared to the Mogollon Rim. First, I grouped individuals into local clusters of samples (sample units) based on geographic proximity alone, without imposing any notions of subpopulation boundaries within each of the two regions. Following McRae et al. (2005) I used a simple hierarchical clustering algorithm (UPGMC, Sneath and Sokal 1973) to group nearest individuals and newly created clusters based on Euclidean distance between individuals and cluster centroids. At each step, a new centroid location representing the geographic center of all individuals in the new, combined cluster was calculated. I allowed individual and clusters to merge up to a maximum distance of 25 km, based on dispersal distance reported for white-tailed deer. Although no data were available on dispersal distance for Coues white-tailed deer, dispersal distance of white-tailed deer in other regions was estimated as 11- 38 km (sexes combined) in Minnesota (Nelson 1993), 2-15 km for yearling males in south Texas (McCoy et al. 2005), and 19.5km for yearling females and 18.5 km for yearling males Wyoming (Dusek et al. 1989).

These sample groups need not represent panmictic populations. I treated these clusters as local sample groups and assumed their allele frequencies represented genotypes in the geographic area (McRae et al. 2005).

I used Microsatellite Tool Kit for Excel to calculate descriptive statistics for sampling clusters including number of alleles per locus, expected and observed

heterozygosity (Table 3). To test whether GMUs corresponded to biologically meaningful boundaries, I used FSTAT (Goudet, J. 2001, version 2.9.3) to calculate multilocus F_{IS} for each cluster group and corresponding GMU. I considered F_{IS} values > +0.10 associated with $P < 0.05$ as indicating a heterozygote deficit within populations.

I measured genetic distance between sampling populations by calculating pair-wise F_{ST} values using FSTAT. Positive F_{ST} values among two populations indicate a deficit of heterozygotes among the two populations and significant values provide evidence of population differentiation. I quantified isolation by distance by running a simple Mantel test (Mantel 1967) using "zt: a software tool for simple and partial Mantel tests" (Bonnet and Van de Peer 2002) with number of iterations ranging from 100,000 to 362,000 and plotting $F_{ST}/ (1- F_{ST})$ as advocated by Rousset (1999) against log-transformed geographic distance. This analysis was conducted to determine how geographic distance explained variation in genetic differentiation.

Linkage Disequilibrium

I used GENEPOP version 3.3 (Raymond and Rousset 1995) to test for linkage disequilibrium (LD) for each locus pair across all populations (Fisher's exact test). I also used GENEPOP to test for significant departures from Hardy-Weinberg equilibrium (HWE) within sample groups. The Markov chain methods with 5,000 dememorization steps, 1,000 batches and 5,000 iterations were used in GENEPOP to test for LD and HWE.

Neighbor joining analysis

I used F_{ST} values to construct a Neighbor joining tree using the NEIGHBOR subroutine in PHYLIP version 3.61 (Felsenstein 1993) and to perform non-metric

multidimensional scaling ordinations (NMDS) on the 23 sample clusters using PRIMER version 5.3 (Primer-E Ltd., Plymouth,UK).

RESULTS

Three-hundred fifty-eight distinct Coues white-tailed deer were identified. No duplicate samples from antler and tissue were discovered. All 26 mule deer samples had genetic profiles inconsistent with Coues White-tailed deer genotypes, increasing my confidence in the lab results. One pair of microsatellite marker loci, OhD and OhP, showed significant linkage disequilibrium (P=0.035; no Bonferonni adjustment) The disequilibrium between OhD and OhP was significant in 5 of 23 sampling clusters (P=< 0.05, with P< 0.01 for only one sampling cluster).

Individual based analysis-estimation of K number of populations

I used the Bayesian clustering algorithm to test for sub-structuring with different values of K (i.e., numbers of populations), ranging from a single panmictic population to 10 populations. Probabilities of the data given the model peaked at K=3 (Table 3). However, the largest improvement in probability occurred between K values of 1 and 2, and there was less improvement between K values of 2 and 3. Most individuals were strongly assigned to one of two populations with K=2 giving a strong indication of real population structure (Prichard and Wen 2003) compared to K=3, in which individuals displayed a more admixed assignment into all three populations. Further, the map for K=2 depicts one sub-population spanning the sky islands and a second sub-population along and below the Mogollon Rim, while the map for K=3 does not correspond as clearly of a geographic pattern (Figure 2a and Figure 2b).

Analysis based on geographic clusters of samples

Based on geographic proximity, the UPGMC method allocated 358 individuals into 23 clusters of ≥ 6 individuals each, 9 in the mainland and 14 in sky islands (Figure 3). There were 5 loci which deviated from Hardy Weinberg Equilibrium (Rt13, Rt24, BM4107, OhD and OhN). Seven clusters were also out of HWE (I-SC1, I-SE, I-SW1, I-SW2, I-N2, I-SC6 and M-N1. This method resulted in cluster groups which closely corresponded to Game Management Unit (GMU) boundaries on mountain ranges in Arizona. Fourteen of 21 Arizona clusters had all their individuals originate from a single GMU. Four clusters had individuals originating from 2 different GMU's, of which one cluster straddled an interstate highway. Two clusters were composed of individuals from 3 different GMU's. Twenty-one samples were not incorporated into clusters. Two of 23 GMU's had high F_{IS} values (Table 5) which indicate a heterozygote deficit within these populations.

Number of alleles per locus ranged between 9 and 24. Average number of alleles per locus (Table 3) varied with the number of individuals in sampling cluster, from a low of 4.2 in I-NW (Catalina Mountains, 6 deer) to a high of 8.0 (Table 3) in I-SC1 (Fort Huachuca, 35 samples). Mean expected heterozygosity within population ranged from 0.62 to 0.73 (Table 3), and mean observed heterozygosity ranged from 0.58 to 0.72.

Non-metric multidimensional scaling (NMDS) plot of 23 clusters (Figure 6) indicated that 8 of 9 Mainland clusters formed a distinct clade, 12 of 14 sky island clusters also formed a distinct clad, with M-NW clustering closer to I-NW and I-SW. Sky island clusters I-N 2, I-NW 1, and I-NW appeared to be intermediate between Mainland and sky island clusters.

Accepting the idea of two distinct sub-populations, one along the Mainland region and one along the sky island region, generated with the program STRUCTURE, I conducted an among-sub-population analysis for the 14 island clusters and a separate analysis for the 9 Mainland clusters.

Isolation by distance

There was a stronger correlation and steeper slope between genetic and geographic distance among the 14 sky island sampling units ($R^2 = 0.372$, $P<0.0025$) than among the 9 Mainland sampling units ($R^2 = 0.201$, $P=0.1499$) (Figure 7). Two pairs of Mainland sampling units M-C2 with M-C1 and M-C2 with M-C3 in close geographic proximity had the 1st and 4th greatest genetic distance of any of the 36 Mainland pairs.

Allelic richness

I observed differences in genetic diversity for Coues white-tailed deer between mainland and sky island regions. A total of 161 alleles were observed in the study area with 143 alleles found in the sky island region and 131 alleles found in the Mainland region. Nearly twice as many unique alleles were found in the sky islands (30) as in the Mainland (18).

Geographic gaps in sample distribution were due to land ownership, with few samples from the San Carlos Apache lands and no samples from White Mountain Apache and Tohono O'odham lands. These gap areas extend along and below the Mogollon Rim between the villages of Young and Hannagan Meadow, Arizona and east of the Baboquivari Mountains southwest of Tucson. Coues white-tailed deer occur and are hunted on these Tribal lands.

DISCUSSION

My results suggest that two subpopulations of Coues white-tailed deer- one along and below the Mogollon Rim and a second among the sky islands of southeastern Arizona and southwestern New Mexico.

The genetic structure of Coues white-tailed deer populations in the sky islands suggests that these woodland specialists are partially isolated by the pattern of non-woodland vegetation. Sullivan (1994) concluded that gene flow of another woodland specialist, the Mexican woodrat (*N. mexicanus*) was similarly affected by late Pleistocene habitat changes in similar southwest United States landscapes because semi-desert grasslands, chaparral and desert scrub functioned as major barriers, reducing gene flow 3-10 times compared to areas separated by woodland. Lomolino et al. (1989) also concluded that desert scrub vegetation in the American Southwest constitutes a very strong barrier to dispersal of small terrestrial and woodland mammals including dusky shrew (*Sorex monticolus)* and long tailed vole (*Microtus longicaudus)*.

Spatial scale and pattern of genetic differentiation

Low F_{IS} values in 21 of the 23 sampling populations support the notion that movement was usually unrestricted within the scale at which the sampling populations were delineated. Paired sampling clusters all occurred in the sky island region of the study area. Of the two sampling unit clusters with high F_{IS} values, I-SC 1 (Fort Huachuca, 26 samples) is the most puzzling. Hunting on Fort Huachuca is divided into separate subunits with an even number of animals harvested in each subunit. No topographic or anthropogenic barriers seem to hinder movement of deer from area to area.

Given the apparent low level of population subdivision, most GMUs apparently do correspond to discrete subpopulations. This supports their usefulness as a means to manage game animals (white-tailed deer). In the southeastern portion of Arizona GMU's correspond to individual mountain ranges.

Low correlation of genetic to geographic distance in the mainland suggests that successful long-distance dispersal and genetic interchange is common along and below the Mogollon Rim and isolation by distance is the mechanism that dominates in this landscape. A higher correlation of genetic to geographic distance in the sky island region suggests the "sea" of desert scrub and desert grassland between most isolated mountain ranges is a barrier to movement by Coues white-tailed deer and vicariant processes are the mechanism for change in this landscape.

Linkage disequilibrium between the OhD and OhP loci would not have been statistically significant if I had used a Bonferroni adjustment, and was evident in only 5 of 23 sampling clusters. Thus I believe that disequilibrium did not compromise the independence of our loci.

Two of the 5 loci in our study that deviated from HWE, OhD and OhN, were also found to deviate from HWE by DeYoung et al. in their study of white-tailed deer (2003). The Rt13 loci which deviated from HWE in our study was also found to deviate from HWE in Polziehn et al. (2000) study of North American wapiti (*Cervus elaphus)* populations. Population substructure, nonrandom mating or population admixture may cause deviations from HWE (DeYoung et al. (2003).

Until Euro-American settlement about a century ago, Coues white-tailed deer of the sky island region may have functioned as a metapopulation, with reduced gene flow among

montane habitats, and occasional recolonization after local extinction. The conversion of agricultural lands to residential development in the valleys between mountain ranges may further fragment the landscape matrix. This fragmentation may reduce movement among habitat patches, increasing extinction rates in local populations and decrease rates of recolonization of vacant patches (Hanski and Gilpin 1991). Brown and Henry (1981) observed Coues white-tailed deer in isolated regions of the Sonoran desert that now no longer support Coues deer. Although tissue samples/data were collected over multiple years, they provide a snapshot of the genetic pattern for Coues white-tailed deer 2001-2003, which forms a baseline that can allow managers and researches to determine whether genetic pattern of Coues white-tailed deer changes in the future due to natural or human-caused alterations in the landscape.

Some of our sampling clusters in the sky islands corresponded to sampling clusters in a study of puma population genetics (McRae 2005). Because both studies used microsatellites and similar number of loci (12 in this study, 16 for puma), pairwise F_{ST} values can be compared between the species. The pair-wise F_{ST} value of 0.034 for puma between McRae's SAZ1 (Baboquivari/Sierrita Mountains) and SAZ2 (Huachuca/Dragoon Mountains) is similar to the 0.028 for Coues deer between our SW1 (Baboquivari/Sierrita Mountains) and I-SC1 (Fort Huachuca Mountains). Most other pair-wise comparisons indicated greater genetic divergence between puma clusters than between corresponding Coues deer clusters. For instance McRae's (2005) SAZ3 (Peloncillo/Chirichua Mountains) diverged by 0.064 F_{ST} units from SAZ1 (Baboquivari/Sierrita Mountains) compared to 0.026 for our I-SE (Animas/Peloncillo Mountains) and I-SW1 (Baboquivari/Sierrita Mountains). This result is somewhat

surprising given the much greater dispersal ability of puma. Perhaps lower population density of puma promotes genetic divergence, more than offsetting increased dispersal ability.

Although I could have delineated population clusters in the sky island region based on GMU boundaries that correspond to obvious habitat patches (individual sky islands), habitat discontinuities were not obvious in the Mainland region. The UPGMC method delineated sampling populations without subjectivity or bias. Low F_{IS} values in 20 of the 23 sub-populations suggest this method was successful in allowing us to estimate average allele frequencies at the cluster centroid.

An unexpectedly high ratio of genetic distance to geographic distance for the sampling clusters M-C1 with M-C2 and M-C2 with M-C3 (corresponding to Game Management Units 24A and 24B, 24A and 23 in Figure 4) may be due to a combination of habitat discontinuities and human alteration of the landscape. Cluster M-C2 (Superstition Mountains) has steep rocky terrain, and a narrow band of desert scrub vegetation separating it from its neighboring two mountain ranges. Mining activity in the Globe-Miami mining district, which began in 1862 and continues today, has created massive wastelands of unvegetated steep tailings, but these create a barrier around only about 20% of the eastern side of the Superstition Mountains. The creation of Theodore Roosevelt, Apache, Canyon and Saguaro Lakes may decrease gene flow between the Superstitions and Mazatzals to the north by forcing dispersing Coues deer to swim across open water or wade thru backwater.

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Table 1. Locus name, species in which they were originally identified, allelic diversity (alleles), size (in base pairs) and reference (Rt, BL, BM and Ovir dinucleotide repeats, Oh tetranucleotide repeats).

Locus	Species of origin	Alleles	Size range	Genebank or reference
Rt5	Rangifer tarandus	22	143-177	U90738
Rt 7	Rangifer tarandus	12	210-234	U90740
Rt 13	Rangifer tarandus	14	262-305	U90743
Rt 24	Rangifer tarandus	16	203-233	U90746
BL 42	Bos taurus	9	240-258	Bishop 1994 Genetics 136:619
BM 4107	Bos taurus	17	135-169	G18519
BM 6506	Bos taurus	9	175-207	G18455
Ovir A	Odocoileus virginianus	15	174-296	L35576
Ovir H	Odocoileus virginianus	11	117-139	L35583
Oh D	Odocoileus hemionus	9	153-189	AF1022
Oh N	Odocoileus hemionus	24	245-313	AF102244
Oh P	Odocoileus hemionus	10	212-244	AF102240

Table 2. PCR primers for 12 microsatellite loci, FAM HEX and TET are fluorescent dyes (Applied Biosystems) used to visualize amplified DNA on a model 310 capillary electrophoresis DNA sequencer.

Marker/label	Sequence	MgCl
BL 42	ACA AGT CAA GGT CAA GTC CAA ATG CC	2
BL 42 TET	CGA TTT TTG TGT TAA TTT CAT GC	
BM4107	ATA GGC TTT GCA TTG TTC AGG	2
BM4107 FAM	AGC CCC TGC TAT TGT GTG AG	
BM6506 L	AAC TTA GCA ACT TGA GCA TGG	1.5
BM6506 U HEX	GTG GTA AAG AGA TGG CAT AGC A	
OheD	TTG CTG CTT GCT TGT CTA AT	1.5
OheD TET	AGA GCC TCG TCT TTT CAT TC	
OheN	Fam GCA ACC AAT AGG ATA GGT CG	1.5
OheN	GCT GGA TGG AAC TGA AAG TC	
OheP HEX	CAG CCT CTA AAA GTT TTC ACT G	2
OheP L	AAT TTG TAA CAT GCC CAA TCA	
Ovir A L HEX	CAC AAA GAA TCA GAC GTG GT	2
OvirA U	G TGC ATC TCA ACA TGA GTT AGG	
OvirH L	AAG TCT ACA ATC CAT GGG CTT GC	1.5
OvirH U TET	GTT CTT TAC CAC CTG CAC CA	
Rt13 L	AT CCC AGA ACA GGA GTG AG	1.5
Rt13 U HEX	AGA GAA TGG CCC AGT GTT AG	
Rt24	GTG TAT CCA TCT GGA AGA TTT CAG	2
Rt24 FAM	CAG TTT AAC CAG TCC TCT GTG	
Rt5 FAM	CAG CAT AAT TCT GAC AAG TG	2
Rt5 L	GTT GAG GGG ACT CGA CTG	
Rt7	ACT TTT CAC GGG CAC TGG TT	2
Rt7 TET	CCT GTT CTA CTC TTC TTC TC	

Table 3. Descriptive statistics for 23 geographically delineated Coues white-tailed groups, including population identifiers (ID), geographic features associated with sample groups (locale), sample size (n), mean number of alleles per locus (Alleles), average expected heterozygosity (H_E), average observed heterozygosity (H_O).

ID	Locale	N	Alleles	Alleles S.D.	H_E	H_O
I-SC 1	Fort Huachuca	26	8.0	2.70	0.734	0.648
I-SC 2	Chiricahua Mountains	13	5.92	2.71	0.641	0.583
I-SE	Animas and Peloncillo Mountains	35	8.67	1.53	0.698	0.651
I-SW 1	Baboquivari Mountains	24	7.17	2.96	0.710	0.684
I-SW 2	Pajarito Mountains	11	6.5	2.39	0.700	0.629
I-SC 3	Santa Rita Mountains	11	6.75	2.21	0.720	0.720
I-SC 4	Whetstone Mountains	13	6.17	2.94	0.693	0.660
I-SC 5	Dragoon and Little Dragoon	12	5.75	2.26	0.690	0.667
I-NW 1	Rincon Mountains	10	5.75	2.52	0.725	0.667
I-N 1	Galiuro Mountains	9	4.58	2.38	0.627	0.611
I-NE	Pinaleno Mountains	13	6.25	2.34	0.726	0.686
I-N 2	Aravaipa	12	5.5	1.68	0.660	0.625
I-SC 6	Mule Mountains	6	4.42	1.71	0.682	0.597
I-NW	Catalina Mountains	6	4.17	2.15	0.654	0.667
M-N 1	Verde River	28	7.58	2.90	0.675	0.610
M-N 2	Mazatzal Mountains	10	5.17	2.54	0.685	0.690
M-E 1	White Mountains	21	6.67	2.09	0.723	0.696
M-C 1	Pinal Mountains	9	5.0	2.48	0.659	0.640
M-C 2	Superstition Mountains	15	5.92	2.42	0.707	0.680
M-C 3	Sierra Ancha Mountains	17	5.83	1.98	0.671	0.671
M-NW	Sycamore Canyon	17	6.75	1.78	0.705	0.655
M-E 2	Gila	11	5.58	1.24	0.693	0.660
M-E 3	Black Range	8	5.08	1.19	0.675	0.667

Table 4. Number of clusters tested (K), probability for the data for each value of K (Ln(P/D)),variance of the probability (Var) and admixture (Alpha).

K	Ln(P/D)	Var	Alpha
1	-14941.2	76.6	-
2	-14731.5	386.8	0.3693
3	-14634.8	643.9	0.3086
4	-14688.6	885.9	0.1889
5	-14690.6	1153.9	0.1457
6	-14661.3	1292.2	0.1414
7	-14704.9	1555.8	0.1203
8	-14981.3	2249.2	0.0919
9	-15142.9	2684.9	0.0769
10	-15685.7	3823.8	0.0708

Table 5. Arizona Game Management Unit (GMU) geographic features associated with sample groups (locale) and F_{IS} indicating heterozygote deficiency.

GMU	Locale	Cluster	F_{IS}
35 A	Fort Huachuca Mountains	I-SC 1	**0.119*****
29	Chiricahua Mountains	I-SC 2	0.093***
30 A	Peloncillo/Animas Mountains	I-SE	0.063
36 C	Baboquivari Mountains	I-SW 1	0.037**
36 B	Pajarito Mountains	I-SW 2	**0.102***
34 A	Santa Rita Mountains	I-SC 3	0.002
34 B	Whetstone Mountains	I-SC 4	0.050
30 B and 32	Dragoon and Little Dragoon Mountains	I-SC 5	0.035*
33	Rincon Mountains	I-NW 1	0.085
32	Galiuro Mountains	I-N 1	0.027
31	Pinaleno Mountains	I-NE	0.058
31, 32 and San Carlos Unit D-West	Aravaipa	I-N 2	0.057*
30 B	Mule Mountains	I-SC 6	0.135
33	Catalina Mountains	I-NW	-0.012
6 A and 21	Verde River	M-N 1	0.098
22	Mazatzal Mountains	M-N 2	-0.008
27 and 28	White Mountains and Blue Range	M-E 1	0.038
24 A	Pinal Mountains	M-C 1	0.031
24 B	Superstition Mountains	M-C 2	0.039
23	Sierra Ancha Mountains	M-C 3	-0.002*
6B and 8	Sycamore Canyon area	M-NW	0.073
24	Pinos Altos area	M-E 2	0.051
16 B and 21 A	Black Range	M-E 3	0.013*

*P<0.05, **P<0.01, ***P<0.001.

Table 6. Pairwise F_{ST} values between 23 Coues white-tailed deer sample clusters in Arizona and New Mexico. Bold values indicate significance at the 0.05 level.

	I-SC 1	M-N 1	M-N 2	I-SE	I-SC 2	I-SW 1	I-SW 2	I-SC 3	I-SC 4	I-SC 5	I-NW1	I-N 1
I-SC 1												
M-N 1	**0.0352**											
M-N 2	0.0082	0.0037										
I-SE	**0.0339**	**0.0527**	0.0225									
I-SC 2	**0.0437**	**0.0472**	0.0155	0.0166								
I-SW 1	**0.0283**	**0.0561**	**0.0285**	**0.0258**	**0.0434**							
I-SW 2	0.0203	**0.0391**	0.0230	0.0253	0.0244	0.0042						
I-SC 3	0.0090	**0.0340**	0.0209	0.0159	0.0382	**0.0294**	0.0225					
I-SC 4	0.0102	**0.0400**	0.0055	0.0063	0.0207	0.0174	0.0149	0.0099				
I-SC 5	**0.0293**	**0.0545**	0.0336	0.0160	0.0188	**0.0417**	0.0484	0.0171	0.0112			
I-NW 1	0.0129	0.0152	0.0069	**0.0312**	**0.0498**	**0.0379**	0.0340	0.0075	0.0079	0.0434		
I-N 1	0.0332	0.0466	0.0193	0.0268	0.0377	0.0375	0.0284	0.0334	0.0084	0.0311	0.0287	
I-NE	0.0141	**0.0297**	-0.0004	0.0277	0.0146	0.0474	0.0415	**0.0241**	0.0186	0.0259	0.0170	0.0208
I-N 2	0.0229	0.0135	0.0131	0.0288	**0.0504**	**0.0478**	0.0412	0.0266	0.0145	0.0411	0.0053	0.0086
M-E 1	**0.0499**	**0.0263**	**0.0444**	**0.0751**	**0.0757**	**0.0726**	**0.0722**	**0.0529**	**0.0588**	**0.0642**	0.0127	**0.0724**
M-C1	**0.0393**	0.0322	0.0120	**0.0255**	**0.0352**	**0.0500**	0.0357	**0.0430**	**0.0218**	**0.0285**	0.0221	0.0312
M-C 2	**0.0446**	0.0182	0.0356	**0.0864**	**0.0896**	**0.0766**	**0.0739**	**0.0593**	**0.0697**	**0.0893**	0.0098	**0.0895**
M-C 3	**0.0407**	0.0062	0.0207	**0.0703**	**0.0730**	**0.0650**	**0.0488**	**0.0499**	**0.0543**	**0.0798**	**0.0196**	**0.0700**
M-NW	**0.0271**	-0.015	0.0050	**0.0490**	**0.0572**	**0.0489**	**0.0448**	0.0235	**0.0362**	0.0440	0.0187	**0.0553**
M-E 2	**0.0449**	0.0229	0.0317	0.0470	0.0711	0.0506	0.0473	0.0204	0.0328	0.0603	-0.0016	0.0560
M-E 3	**0.0565**	**0.0212**	0.0480	**0.0709**	0.0797	**0.0818**	0.0756	0.0381	**0.0661**	**0.0592**	0.0178	0.0840
I-SC 6	0.0351	**0.0699**	0.0430	**0.0425**	0.0267	0.0378	0.0301	0.0326	0.0164	0.0375	0.0362	0.0387
I-NW	0.0264	0.0147	0.0238	0.0235	**0.0531**	0.0508	0.0613	0.0120	0.0246	0.0462	-0.0177	0.0478

Table 6. Continued.

	I-NE	I-N 2	M-E 1	M-C 1	M-C 2	M-C 3	M-NW	M-E 2	M-E 3	I-SC 6	I-NW
I-NE											
I-N 2	0.0180										
M-E 1	**0.0413**	**0.0391**									
M-C 1	0.0244	0.0232	**0.0494**								
M-C 2	0.0508	**0.0358**	0.0151	**0.0700**							
M-C 3	**0.0529**	**0.0345**	**0.0352**	**0.0501**	0.0108						
M-NW	0.0297	0.0182	**0.0414**	0.0302	0.0308	0.0082					
M-E 2	0.0514	0.0391	0.0170	0.0406	0.0149	0.0226	0.0251				
M-E 3	**0.0427**	**0.0474**	0.0095	0.0601	0.0248	0.0212	0.0316	-0.0024			
I-SC 6	0.0167	0.0514	**0.0662**	**0.0407**	0.0764	**0.0845**	0.0619	0.0605	0.0760		
I-NW	0.0265	0.0054	0.0339	0.0366	0.0112	**0.0243**	0.0198	0.0153	0.0208	0.0694	

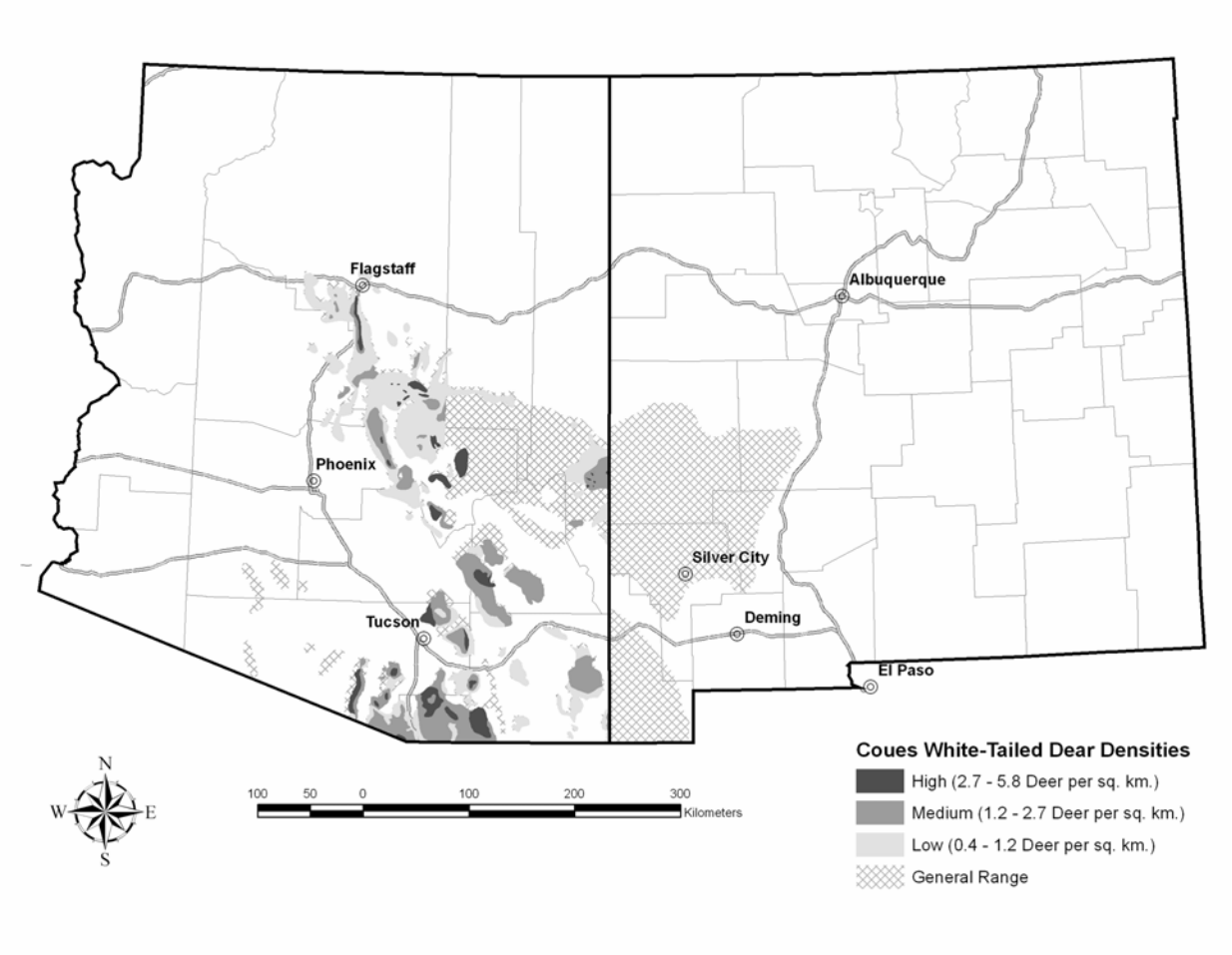

Figure 1. General distribution and density for Coues white-tailed deer in Arizona and New Mexico. Adapted from Ockenfels 1991, J. Heffelfinger and D. Semmens: by J. Jenness.

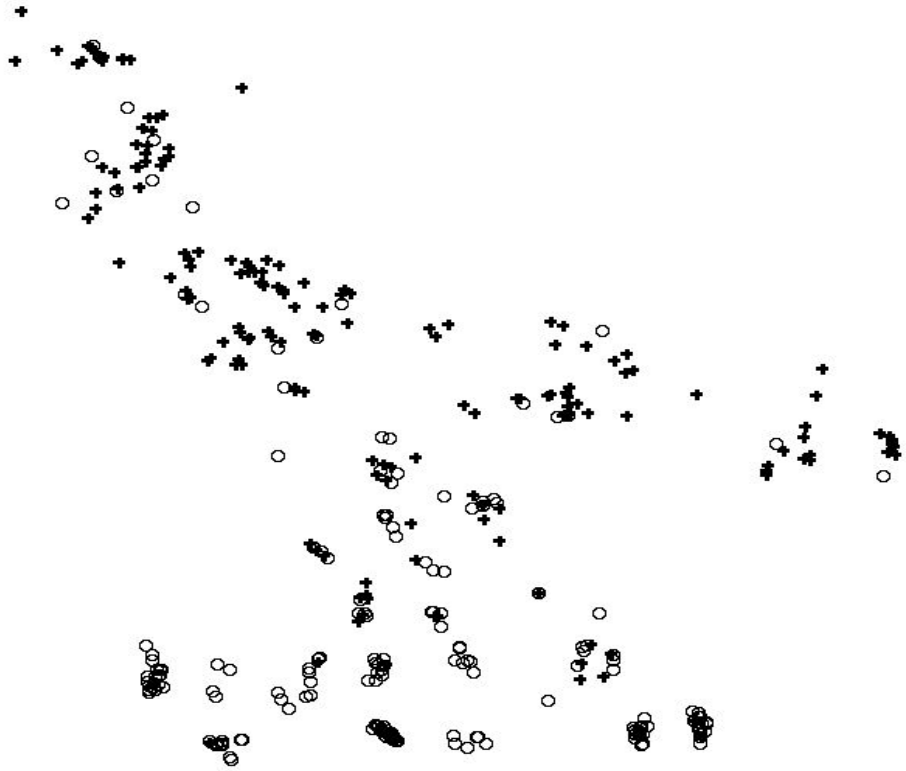

Figure 2a. Results of Program STRUCTURE with K=2 populations corresponding to a Coues white-tailed deer Mainland population (+) and an Islands population (○) in Arizona and New Mexico.

Figure 2b. Results of Program STRUCTURE with K=3 populations (○, ▲, and +) for Coues white-tailed deer in Arizona and New Mexico.

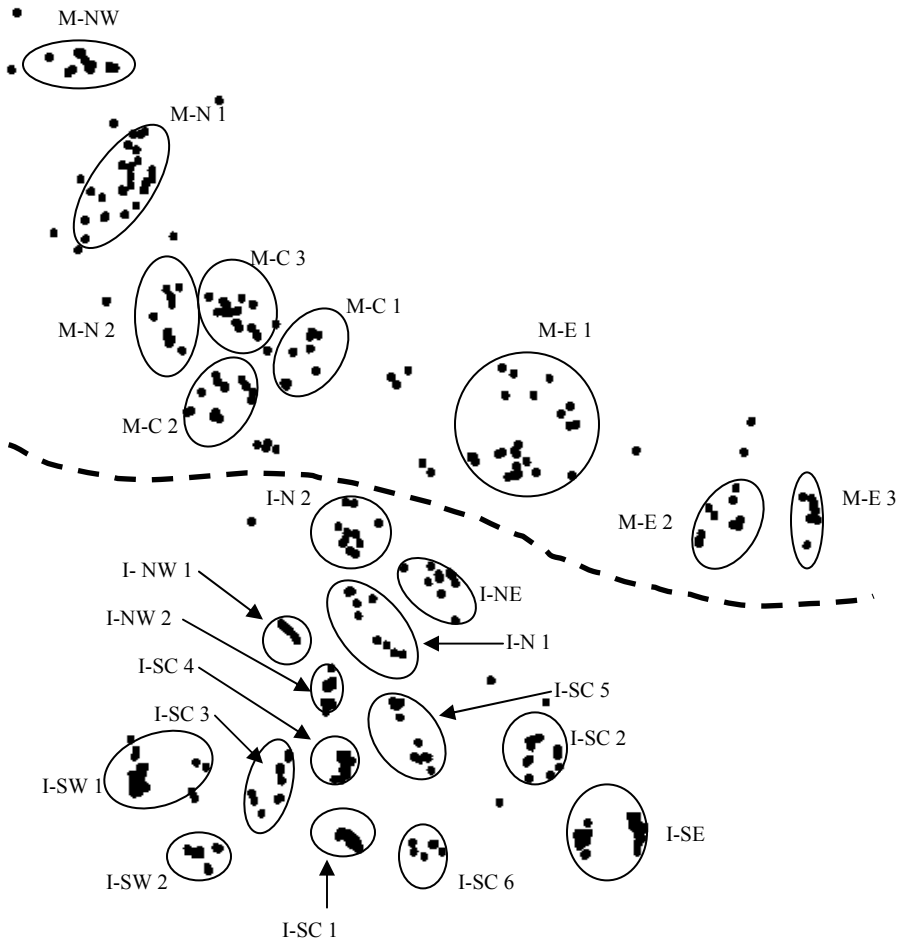

Figure 3. Ellipses indicate results of sampling cluster with 337 Coues white-tailed deer forming 23 clusters in Arizona and New Mexico. Nine clusters formed in the Mainland region and 14 clusters formed in the sky islands region. Identification numbers correspond to labels in Table 2. The dashed line indicates the dividing line between the 2 sub-populations indicated in Figure 2 A.

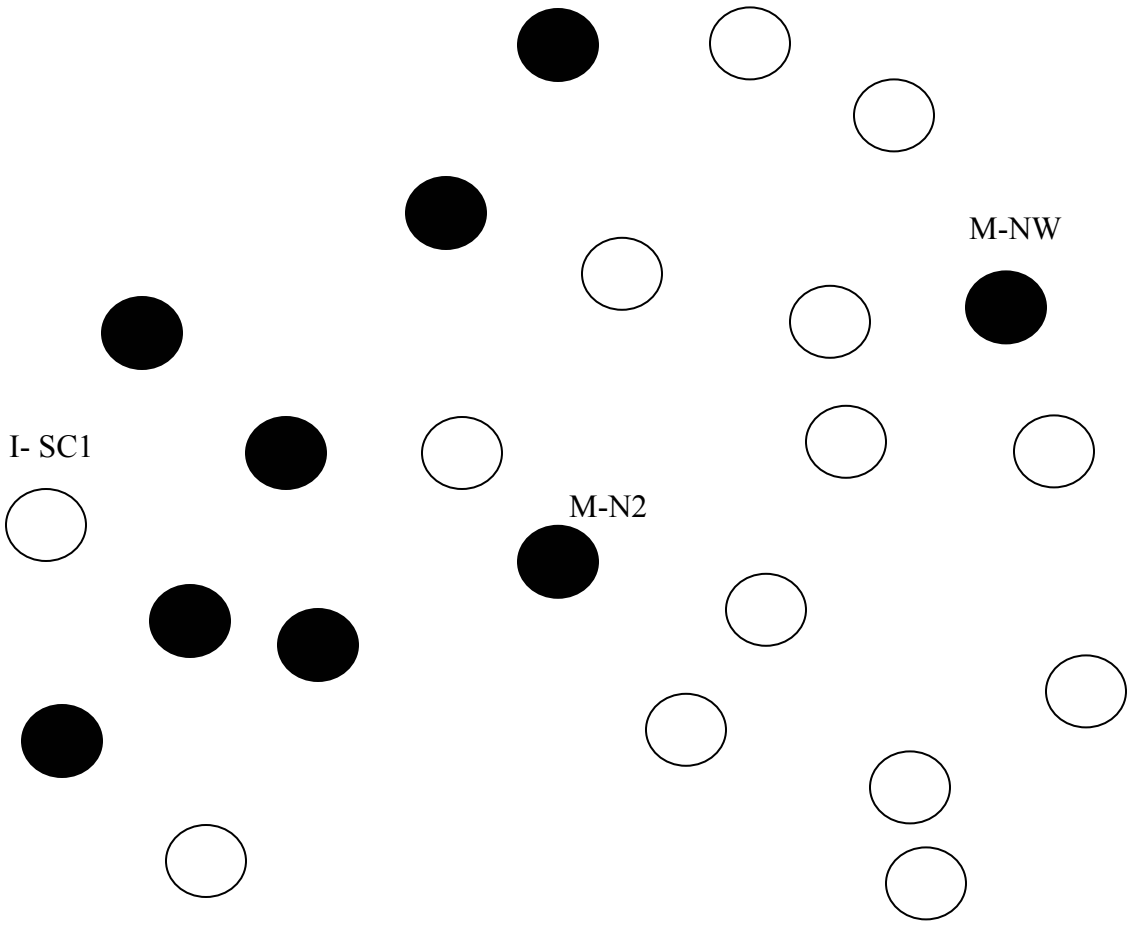

Figure 4. Non-metric multidimensional scaling plot of 23 white-tailed deer clusters in Arizona and New Mexico. Closed circles indicate clusters north of dividing line in Figure 3 (Mainland populations) and open circles indicate clusters south of the dividing line in Figure 3 (Sky island populations).

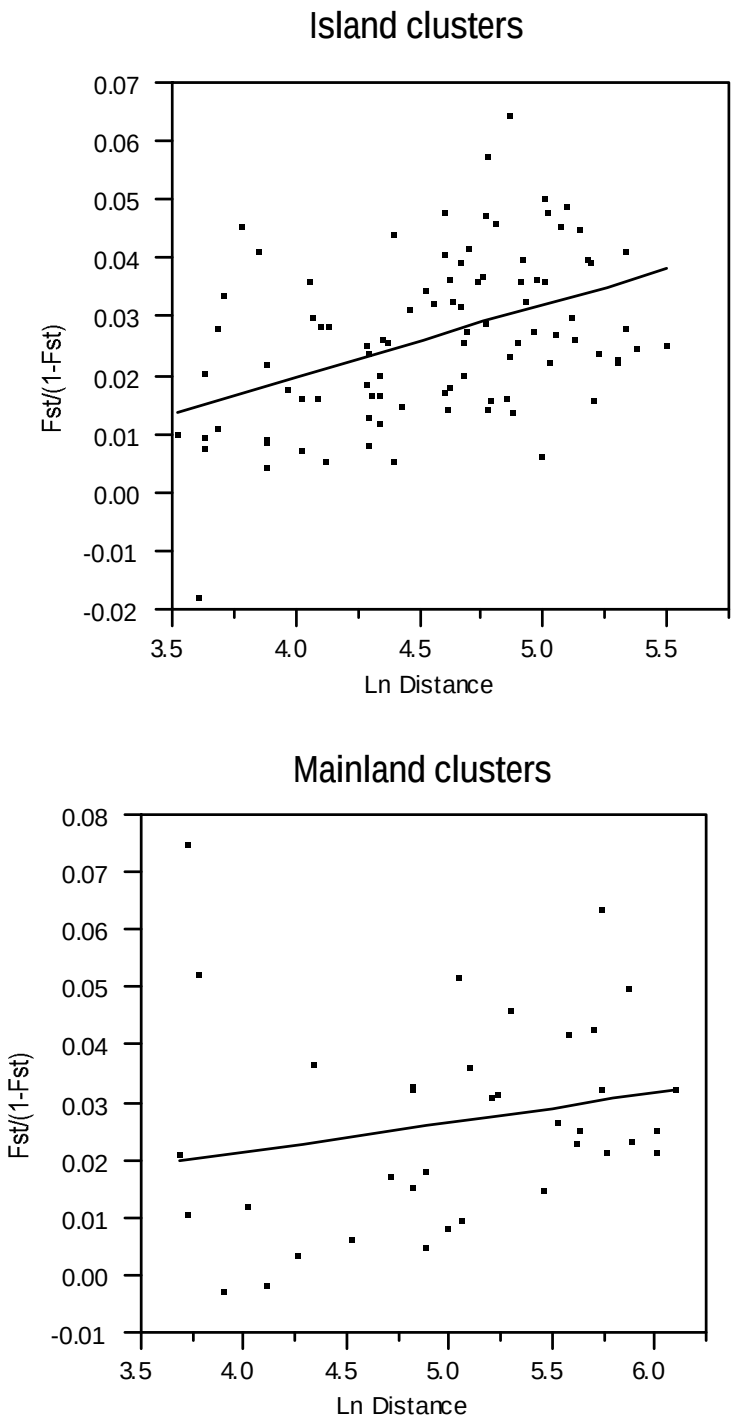

Figure 5. Coues white-tailed deer genetic (F_{ST}/1- F_{ST}) as a function of geographic distance (Ln km) for sky island and Mainland clusters in Arizona and New Mexico.

Chapter 2

HYBRIDIZATION RATE BETWEEN COUES WHITE-TAILED DEER AND MULE DEER IN ARIZONA AND NEW MEXICO

Abstract: I used microsatellite DNA markers to investigate the extent of natural hybridization between Coues white-tailed deer (*Odocoileus virginianus couesi*) and desert mule deer *(O. hemionus eremicus)* in Arizona and New Mexico. Our objective was to determine how commonly this occurs in wild populations of Coues white-tailed deer in the southwestern United States. I used admixture analysis and historic tissue samples of known captive-raised hybrid Coues white-tailed and mule deer. Tissue samples in the form of muscle, skin and cast antler from 365 purported Coues white-tailed deer, 19 purported mule deer, 4 purported Coues/mule deer hybrid, from Arizona and New Mexico were genotyped. Additionally, tissue samples from 7 wild deer suspected to be Coues/mule deer hybrids were analyzed. Using a Bayesian clustering method STRUCTURE, 358 Coues white-tailed deer, 26 mule deer and 10 hybrid Coues/mule deer genotypes were identified. Our results suggest about 2% of wild free ranging Coues white-tailed deer in Arizona and New Mexico are hybrids, including some animals that are probably backcrosses to each parental species.

INTRODUCTION--Hybridization is most commonly used to describe the successful reproduction of two individuals belonging to different species (Gray 1972). Species are prevented from interbreeding by a variety of means. They may not occupy the same area at the same time or they may possess behavioral differences, usually in courtship behavior, so that one species does not recognize the visual, auditory, or olfactory mating cues of the other species (Heffelfinger 2000). Natural hybridization in wild populations of plants, fish, birds, and insects has been documented for many years. Knobloch (1972) purported 26,000 instances of hybridization between species or genera of angiosperms. Although Knobloch (1972) listed many artificial hybrids (Stace 1975) the list included thousands of bona fide natural hybridizations (Arnold 1997). In contrast to plants, hybridization between animal taxa is "rare" (Arnold 1997) but does occur as with saltwater (Nielsen et al. 2003) and freshwater fish (Allendorf and Leary 1988), Africanized and European honeybees (Rabe et al. 2005), birds, and canids (Wayne and Jenks 1991; Roy et al.1994)

Hybrids of white-tailed deer (*Odocoileus virginianus*) and mule deer (*O. hemionus*) have been successfully bred in captivity (Nichol 1938; Cowan 1962; Whitehead 1972; Day 1980; Wishart 1980; Lingle 1992), although interspecific hybridization in most natural populations appears to be rare. Nichol (1938) produced Coues white-tailed/mule deer hybrids from male Coues white-tailed deer mating with female mule deer and the reciprocal male mule deer mating with female Coues white-tailed deer. Hybrid white-tailed X mule deer have also been confirmed from wild free ranging populations in areas where they are sympatric using both morphological and genetic characteristic (Heffelfinger 2000). Using morphological characteristics, Kramer

(1973) reported 2 out of 983 deer from Kansas, and 6 out of several thousand deer observations in Alberta were hybrids. Knipe (1977) observed only 8 definite hybrid deer in 34 years of fieldwork in Arizona based on morphological characteristics. Day (1980) examined over 200 white-tailed and mule deer and only 1 was a hybrid based on morphology while all purported hybrids that he examined were variants of either mule or Coues white-tailed deer. Other documented areas and numbers of white-tailed X mule deer hybrids include 5 in Montana (Cronin 1991) using serum albumin and mitochondrial DNA, 21 in Texas (Stubblefield et al.1986, Bradley et al. 2003) using serum albumin, ribosomal and mitochondrial DNA, 6 in Washington (Gavin and May 1988) using allozyme data, and 13 from western Wyoming (Kay and Boe 1992) using morphological characteristics.

Stubblefield et al. (1986) examined albumin allozymes of 319 deer from 5 counties in the Trans-Pecos region of Texas and concluded that hybridization averaged 5.6% (range 0.0-13.8%) within the 5 counties. Also in the Trans-Pecos region of Texas, Carr et al. (1986) indicated that some specimens identified as mule deer based on morphological appearance had mitochondrial DNA normally associated with white-tailed deer. In Arizona, Derr (1991) found one white-tailed deer with mule deer albumin among 7 Coues white-tailed deer sampled, no Coues white-tailed deer albumin was found among 8 mule deer sampled.

Due to the low survival rate of hybrid fawns in captivity (Nichol 1938; Day 1980), the extent of hybrid Coues white-tailed and mule deer in wild free ranging populations of the American southwest is expected to be low.

The species can be distinguished morphologically by the form of the antler of males and by the size and location of metatarsal gland in both sexes. A white-tailed deer antler consists of a single main beam, whereas in mule deer the antler is forked into two equal branches. As antlers are secondary sex characteristics of adult bucks, the metatarsal gland is an important character in the identification of does and juveniles. The only morphological characteristics that can be used to determine if a deer is a hybrid are size and location of the metatarsal gland (Heffelfinger 2000). The metatarsal glands on mule deer are high on the lower hind leg, 75- 150 mm long, and usually surrounded by brown fur only (Cowan 1956). The whitetail's metatarsals are at or below the midpoint of the lower hind leg, less than 38 mm, and always surrounded by white hair (Cowan 1956). A white-tailed deer X mule deer hybrid has metatarsal glands intermediate in length, measuring 50 - 100 mm and sometimes encircled with white hair (Day 1980). While morphological characteristics can be used to recognize F_1 hybrids, these characteristics can be eroded in backcrossed individuals, with metatarsal gland length reverting to a gland length of the predominant parental source (Day 1980). Genetic evidence of hybridization is retained during backcrossing and may provide the only evidence of F_2 and F_3 hybrids.

My objective was to determine how commonly this occurs in wild populations of Coues white-tailed deer. I used admixture analysis and historic tissue samples of known captive-raised hybrid Coues white-tailed and mule deer. I used microsatellite (nDNA) markers to investigate the extent of natural hybridization between Coues white-tailed deer (*O. v. couesi*) and desert mule deer *(O. h. eremicus)* in Arizona and New Mexico.

METHODS--I collected tissue samples from 365 purported Coues white-tailed deer, 19 purported mule deer, 4 purported Coues/mule deer hybrid, and 7 deer suspected to be Coues X mule deer hybrids. DNA was extracted and genotyped using tissue samples consisting of muscle, skin and antler shavings. Muscle and skin samples were from legally hunted deer and antler samples were from naturally cast antlers that were collected in the field. All samples were from Southwest New Mexico and Central and Southeast Arizona. Hunter harvested tissue samples were collected between November 2001 and December 2003. Antler tissue samples were from antlers that were naturally cast between approximately May 1993 and May 2003. Known hybrids tissue samples were obtained from the School of Natural Resources mammal collection at the University of Arizona, where two complete Coues white-tailed X mule deer hybrid specimens were stored. These two animals resulted from captive Coues white-tailed and mule deer from a deer hybridization study (Day1980) conducted by the Arizona Game and Fish Department in the late 1960's and early 1970's. Skeletal remains of one male and one female are stored in the collection, but no records were available as to what combination of Coues white-tailed or mule deer sire and dam were used to produce the hybrids. The entire skeletons (except antlers) in the University of Arizona mammal collection were stored in separate boxes, and records were absent on how the specimens were prepared (boiled in water, if chemicals were used or if beetles *(Dermestid* spp). were used).

A tissue sample was extracted using a small (8 mm) drill bit which was drilled into the pedicle bone of the male. Drilling was at low revolutions per minute to avoid scorching the sample. Flame sterilization of the drill bit was performed between bone samples (O'Connell and Denome 1999). The shavings were collected on a clean sheet of

paper and placed into an individually marked sterile 2ml vial. A second sample was extracted from the femur of the male and placed in a separate 2ml vial. Two samples were also collected from the female and stored in separate 2ml vials, one from the skull and one from the femur. Additionally, Gerald Day (Arizona Game and Fish Department-retired) provided an antler sample from a 3rd hybrid animal produced in captivity (Day 1980) and tissues from a hunter-harvested deer identified as a Coues white-tailed X mule deer hybrid by serum albumin and erythrocyte acid phosphatase starch gel electrophoresis (P.Dratch, U.S. Fish and Wildlife Service Forensics Lab, personal communication). Tissue samples were sent to Wildlife Genetics International for DNA extraction and individual genotyping. Genotyping was performed using a suite of 12 microsatellite genetic markers that were developed for use in white-tailed deer. A check for identical samples, using Microsatellite toolkit, was conducted in the event that two or more samples represented 1 animal.

DATA ANALYSIS--*Hybridization Analysis*-- I used a Bayesian clustering method (STRUCTURE version 2.3.1, Pritchard et al. 2000) to infer the number of populations and assign individuals to populations based only on multilocus genotype data (i.e., without knowledge of morphology or sample origin). The program STRUCTURE probabilistically assigns individuals to a given population or jointly assigns them to two or more populations if the genotype shows evidence of admixture (Pritchard et al. 2000). Using STRUCTURE I estimated individual admixture proportions, (i.e. the estimated proportion of an individual's genotype originating from one or the other of the parental populations). STRUCTURE is a model-based Bayesian, Markov Chain Monte Carlo

approach that clusters individuals to minimize Hardy-Weinberg disequilibrium. I used an admixture model (i.e., allowing the genetic composition of individuals to be a mixture from different populations) that allowed correlated allele frequencies. All run lengths were 500,000 Markov Chain Monte Carlo and 500,000 for burn-in, the number of simulation runs before collecting data to minimize the effect of the starting configuration, (Prichard and Wen 2003) and 500,000 cycles after burn-in. Using the program STRUCTURE with K=2, Coues white-tailed deer, mule deer and known Coues white-tailed X mule deer hybrid genotype data were analyzed, resulting in the likelihood that an individual genotype came from one of two populations

RESULTS --As expected, the program STRUCTURE suggested that our samples consisted of two populations. Almost all (356 of 365) purported Coues deer samples assigned with > 91% probability to one population as defined by STRUCTURE, and all 19 purported mule deer samples assigned with >99% to the other population (Table 1). Hereafter I refer to these 2 genetically distinct populations as inferred Coues deer and inferred mule deer, respectively. Two flesh/hide samples submitted by hunters as Coues white-tailed deer and three cast antlers submitted as Coues white-tailed deer were assigned > 0.99% probability to the inferred mule deer population, and thus were apparently misidentified by the collectors. Of 7 suspected hybrids submitted by hunters, 3 had genetic assignments consistent with hybrid status, but 2 were assigned to the inferred mule deer population and 2 were assigned to the inferred Coues deer population. Both tissue samples from the University of Arizona female hybrid yielded DNA, both tissue samples

from the University of Arizona male failed to yield DNA due to the degraded nature of the tissue sample.

CRYPTIC HYBRIDS DISCOVERED--All 3 known hybrids, 2 of the 7 suspected hybrids, and one sample submitted as a Coues deer assigned into the two populations in the approximate ratio 0.45-0.55 (Table 1), consistent with F_1 hybrid status. One suspected hybrid assigned with 73% probability to the Coues deer and one sample submitted as a Coues deer assigned with 85% probability to the inferred mule deer population suggesting that F_1 hybrids had backcrossed into each parental population. The 90% confidence interval on the latter sample barely overlapped 0.50 which could also be consistent with F_3 hybrid status. Two samples submitted as Coues white-tailed deer assigned with 88% probability to the inferred Coues deer population with confidence intervals that did not overlap 0.50 suggesting they may have been F_3 backcrosses. Genotype data indicated that 358 Coues white-tailed deer, 26 mule deer and 10 hybrid Coues/mule deer were identified.

Of the twelve microsatellite markers, RT5 showed the most consistent differences between Coues and mule deer. RT5 had an even length number of base pairs for 100% of inferred mule deer and an odd length number of base pairs for 96% of inferred Coues white-tailed deer (Table 2). When an inferred Coues deer had an even number of base pairs at the RT5, only one locus was even-length. Nine of the 10 inferred hybrids were likely to have an odd and an even number of base pairs at RT5, the remaining inferred hybrid had odd lengths at both alleles.

DISCUSSION--Our data suggest that about 2% of wild free ranging Coues white-tailed deer in Arizona and New Mexico are hybrids, including some animals that are probably

backcrosses to each parental species. Although the existence of hybrids has been well-known for some time, our study is the first to estimate introgression of mule deer genes into Coues white-tailed deer, and to suggest F_2 and plausible F_3 hybrids between these species. The wild hybrids occurred across the geographic range of Coues white-tailed deer but may be more common in areas of relatively low density of Coues white-tailed deer (Figure 1). Of 7 wild hybrids in Arizona, (the only state for which a map of estimated deer density was available) 5 occurred in areas of low Coues white-tailed deer density and two occurred in areas of medium Coues white-tailed deer density and none occurred in areas of high deer density. Three hybrids were taken in the sky island ecoregion where Coues deer distribution is naturally fragmented by intervening desert habitat, and five 5 hybrids were from the Mogollon Rim area where forest cover is relatively continuous.

Cross–species amplification of the RT5 marker by Wilson et al. (1997) produced a range in base pairs 151-175 for white-tailed deer (n=16) and 146-167 for mule deer (n=17). The allele frequencies I observed for Coues white-tailed deer (base pair range 143-177) cover a somewhat broader range than those sampled by Wilson but similar for desert mule deer (range 146-168). This difference in allele frequencies between subspecies of white-tailed deer suggests that the RT5 marker may be useful in distinguishing between white-tailed deer subspecies and between white-tailed and mule deer and likely hybrids.

Previous research suggests that F_1 and F_2 hybrids have low neonatal survival (Nichol 1938; Day 1980) and that the "confused" escape gait of hybrids (Lingle 1992) may lower survival throughout life. However, our apparent documentation of $F_{2\text{ and }}F_3$

hybrids in the wild suggests that wild hybrids sometimes survive to reproductive age, and are fertile. Metatarsal gland length has been a diagnostic characteristic in identifying hybrid white-tailed and mule deer, but this characteristic can be eroded with backcrossed individuals which would have a metatarsal gland length more similar to the predominant parental species than to an F_1 hybrid.

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Table 1. Probability of individual genotypes assigning into one of two populations, as estimated by program STRUCTURE for deer tissue samples collected in Arizona and New Mexico.

Inferred group based on genetic assignment	Probability of Assignment to	
	Population 1	Population 2
Coues white-tailed deer (n = 358, including 356 samples submitted as Coues deer, 2 identified by hunters as possible hybrids.	Mean 0.993 Range 0.912-0.998 SD 0.01166	Mean 0.007
Mule deer (n = 26, including 19 samples submitted as mule deer, 2 muscle and hide samples identified by hunters as Coues deer, 3 cast antlers submitted as Coues deer, and 2 samples identified by hunters as suspected hybrids)	Mean 0.003	Mean 0.997 Range 0.991-0.998 SD 0.00169
F_1 Hybrid #1 (known female hybrid – Day 1980)	0. 41 (0.19, 0.64)[a]	0. 59 (0.36, 0.81) [a]
F_1 Hybrid #2 (known male hybrid "Donny" – Day 1980)	0. 49 (0.27, 0.70)[a]	0. 51 (0.30, 0.73) [a]
F_1 Hybrid #3 (Alvarez male identified as hybrid by serum albumin)	0.56 (0.31, 0.79)[a]	0.44 (0.21, 0.69) [a]
F_1 Hybrid #4 (submitted as Coues deer)	0.56 (0.34, 0.79) [a]	0.44 (0.21, 0.66) [a]
F_1 Hybrid #5 (submitted as suspected hybrid)	0.51 (0.31, 0.76) [a]	0.49 (0.24,0.69) [a]
F_1 Hybrid #6 (submitted as suspected hybrid)	0.60 (0.37, 0.81) [a]	0.40 (0.20, 0.63) [a]
F_2 Hybrid #7 (submitted as Coues deer)	0.73 (0.54, 0.95) [a]	0.27 (0.05, 0.46) [a]
F_3 Hybrid #8 (submitted as suspected hybrid)	0.15 (0.00, 0.52) [a]	0.85 (0.48, 1.0) [a]
F_3 Hybrid #9 (submitted as Coues deer)	0.88 (0.66, 1.0) [a]	0.12 (0, 0.34) [a]
F_3 Hybrid# 10 (submitted as Coues deer)	0.88 (0.71, 1.0) [a]	0.12 (0, 0.30) [a]

[a] Numbers in parentheses indicate 90% Confidence Interval.

Table 2. Number of deer tissues samples with each of 28 alleles at the RT5 locus. The 16 alleles with an even number of base pairs in Coues white-tailed deer (bold font) occurred in 16 individual Coues deer.

Allele (base pairs)	Inferred Coues deer	Inferred Mule deer	Inferred hybrid
Odd-numbered lengths			
143	109	0	0
145	12	0	0
149	10	0	1
151	2	0	0
153	90	0	0
155	2	0	1
157	37	0	1
159	23	0	0
161	26	0	0
163	4	0	0
165	261	0	4
167	12	0	0
169	37	0	0
171	38	0	1
173	11	0	1
175	22	0	2
177	4	0	0
Even-numbered lengths			
146	0	5	0
148	0	1	0
150	0	4	1
152	**4**	15	3
154	**2**	7	0
156	0	1	2
158	**7**	12	2
160	**1**	6	0
164	**2**	0	0
166	0	1	0
168	0	0	1
All	716[a]	52[a]	20[a]

[a] Numbers of alleles are twice the numbers of sampled animals (358 inferred Coues deer, 26 inferred mule deer, and 10 inferred hybrids).

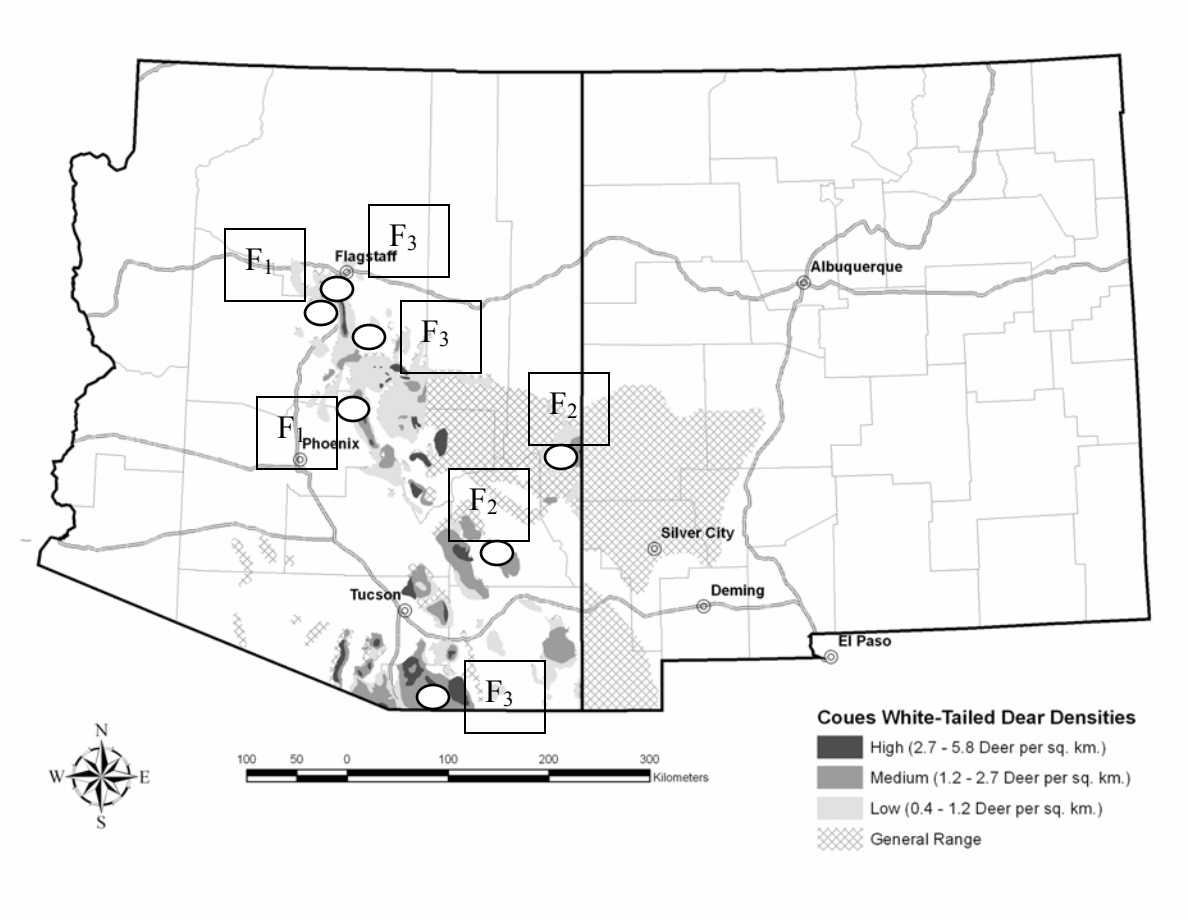

Figure 1. Locations of F_1, F_2 and F_3 Coues white-tailed X mule deer hybrids in relation to distribution and density of Coues white-tailed in Arizona and New Mexico. (Adapted from: Ockenfels et al. 1991, J. Heffelfinger and D. Semmens: by J. Jenness.

CHAPTER 3

WEATHERED ANTLERS AS A SOURCE OF DNA

Abstract: Cast antler in various stages of natural decomposition were tested to determine the amount of weathering cast antlers could endure and still yield usable DNA. Antlers were made available by shed antler collectors who collected cast antlers from Arizona and New Mexico. Seventy-six Coues white-tailed (*Odocoileus virginianus couesi*) and 13 mule deer (*O. hemionus*) cast antlers were tested. Twenty-three Coues white-tailed and mule deer skulls with attached antlers were also tested for usable DNA. Based on physical characteristics of cast and attached antlers, they were placed into seven weathering classes ranging from freshly cast and not subjected to excessive heat and moisture while lying in the field to antlers estimated at having been exposed to 8-10 years of weathering in the field. Antlers were tested by extracting a small amount of shaved antler material using a small diameter drill bit, which was flame sterilized between samples. Most cast antlers in weathering class 1-5 yielded (estimated one to seven years of exposure to sun, moisture and temperature fluctuations) DNA by polymerase chain reaction (PCR). Antlers in weathering class 6 and 7 (an estimated 8 + years of exposure to the elements) failed to yield replicable DNA. Scorching caused by high drill speed during extraction may have caused some antlers, which were in weathering class 1-4, to fail to yield DNA.

Introduction

Studies of population genetics rely mainly on tissues from living or freshly-killed specimens, or from conserved tissues. After an organism dies, its DNA normally becomes degraded by endogenous nucleases, enzymes that cleave nucleic acids and break apart the DNA molecule. Nuclease enzymes can themselves be destroyed or inactivated by rapid desiccation, low temperatures or high salt concentrations (Hofreiter et al. 2001). DNA extraction and sequencing has been performed from ancient bone samples ranging from 4 years to greater than 50,000 years old (Höss et al. 1996). For specimens collected under controlled conditions and stored in museums for up to 200 years, retrieval of DNA sequences using polymerase chain reaction has become routine (Hofreiter et al. 2001). Such samples are usually protected soon after collection from environmental factors (heat, moisture, sunlight) that would degrade DNA. Unlike soft tissue which degrades rapidly after an organism dies, cast antlers, or antlers attached to skulls in private collections, offer a potentially useful source of DNA for such studies.

Antlers are bony protuberances developed on the frontal bones of the skull in most species of deer (Goss 1983). They are normally produced only by males and are cast after the breeding season and regenerated each year (Lincoln 1992). Some cast antlers are gathered by antler collectors, often after several years in the field during which they are gnawed on by rodents and weathered by sunlight and precipitation. After a year or two of exposure to the sun, antlers bleach to a white color, making them more detectable to antler collectors (J. Epperson, Rimrock Outfitters, personal communication). In this study I investigated whether cast antlers of varying degrees of

natural weathering can be used as an alternate source of DNA, substituting for hide, flesh and muscle tissue.

Methods

Antlers were made available by shed antler collectors who collected cast antlers from Arizona and New Mexico. A sub-sample of available Coues white-tailed (*Odocoileus virginianus couesi*) deer and mule deer (*O. hemionus*) cast antlers was tested to determine the amount of natural weathering cast antlers could endure and still yield DNA. Antlers were cast and collected approximately between 1993 and 2002 from southwestern New Mexico, central and southeast Arizona. I tested cast antlers from seventy-six Coues white-tailed deer, ten mule deer and antlers attached to skull plates from twenty-three Coues white-tailed and mule deer.

I extracted a small amount of shaved antler material by drilling an 8mm drill bit into the base of the antler (next to the pedicle bone) 1-2 mm discarding these shallow shavings (O'Connell and Denome 1999), then drilling was continued to a depth of approximately 2-3 cm and collecting the deeper shavings on a clean sheet of paper and transferring them to individually labeled 2 ml vials. The drill bit was flame sterilized between antler samples (O'Connell and Denome 1999). Drill speed was at low revolutions per minute to avoid scorching the antler shavings. For antlers attached to skulls, I drilled into the base of the antler through the skull plate towards the pedicle bone with the skull material being discarded. In 4 cases where the owner of a skull and attached antler was unwilling to allow the antler to be drilled, samples were extracted directly from the pedicle bone not the antler. For cosmetic reasons a site on the backside of the pedicle bone was chosen.

Genetic Analysis

Microsatellite DNA loci are tandemly repeated sequences composed of repeat units (2-5 bases) flanked by unique sequences that are dispersed throughout the mammalian genome (Tautz and Renz 1984, Weber and May 1989). Microsatellite markers are common in eukaryotic DNA and can be amplified by the polymerase chain reaction (PCR), which allows the use of minute or degraded samples (Hughes and Queller 1993). Microsatellite DNA loci have been characterized for several species of large mammals including white-tailed deer (DeWoody et al.1995, Anderson et al. 2003) and mule deer (Jones et al. 2000). Wildlife Genetics International, (Nelson, British Columbia Canada) scored my samples at 12 microsatellite genetic markers.

Weathering class of antlers – Based on the physical weathering of antlers and other evidence, such as finding an antler one year and finding its match in subsequent years, the antler collectors concluded that antlers could be placed into 7 weathering classes (Table 1). Cast antlers in weathering class 1-5 may correspond to years of weathering under modal exposure to the elements. However, there are no quantitative data to calibrate weathering classes to years of exposure, nor even to confirm the reasonable assumption that weathering classes represent a continuum from brief to long duration and intensity or exposure to elements. Most antlers were collected by myself (15 years of experience collecting cast antlers) and 3 antler collectors from New Mexico, central and southern Arizona with 18, 20 and 25 years of experience.

Results

Most cast antlers in weathering class 1-5 yielded useable DNA. Antlers in weathering class 6 and 7 failed to yield DNA. These weathered antlers had characteristic large cracks along the entire length of the antler, a brittle/crumbly exterior, and a pinkish color probably caused by bacterial growth (Bonnie Yates, United States Fish and Wildlife

Service Forensics Lab, personal communication). One set of antlers that had been on top a barn for 8-10 years in southern Arizona had pedicle bone shaving collected twice in an attempt to extract DNA. Both attempts failed; the material was degraded beyond use. Antlers (n=7) that were in weathering class 1-4 and failed to yield DNA may have had the antler material scorched during extraction due to too high of drill speed.

Discussion

This study confirms that cast antlers collected from the field that were in weathering class 1-5, approximately corresponding to 1-5 years of seasonal cycles of moisture and temperature fluctuations, yielded replicable DNA. Samples that were in weathering class 6 and 7, approximately corresponding to more than 7 years of exposure to the elements, did not yield DNA. Climate in the collection area is characterized by a monsoon pattern of summer and late winter precipitation, with pronounced seasonal and diurnal temperature fluctuations.

Tappen (1994) concluded that weathering and crack formation on bones was slower in a rainforest environment than in open savannas of Zaire. Behrensmeyers (1978) concluded that exposure to sunlight and rain and extreme fluctuations in these factors, increased weathering rate of individual bones in Kenya. The mountainous areas where we obtained antlers may experience similar fluctuations in temperature and moisture. Microsites with less solar exposure and less extreme fluctuations are thus probably likely to yield useable cast antlers.

Antler gnawing by rodents can influence the persistence of cast antlers in the field by accelerating the weathering of antlers by exposing the core of the antler. Rodent gnawing can also decrease the presence of cast antlers by consumption of cast antlers. Rodents probably gnaw antler and bones to satisfy mineral intake requirements or to

maintain continuously growing, open-rooted incisors (Poole, 1940). Most antlers exposed to the environment greater than 1 year had evidence of gnawing. Gnaw marks have been discovered on early Pleistocene cervid antlers (Kaiser and Croitor 2004).

Cast antlers are collected by many people for a variety of reasons, ranging from selling cast antlers for profit to collecting cast antlers for display. Many individuals who collect cast antlers record the date and location where the antler was found, and experienced collectors may be able to recognize cast antlers from the same individual in multiple years. Cast antlers from such collections can be an important source of genetic material. Antler shavings are easier to store without spoilage and easier to ship than flesh and hide shipped in ethanol, which is considered a flammable liquid by some transport companies.

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Table 1. Weathering of antlers and number of loci amplified for Coues white-tailed and mule deer in New Mexico and Arizona.

Weathering class	Microsatellite loci amplified												
	12	11	10	9	8	7	6	5	4	3	2	1	0
1	29		1				1		1				1
2	27	1							2				1
3	17	2											
4	12												1
5	3							1		2			1
6	4												
7											2		3

Table 2. Characteristics of naturally weathered cast antlers and skull of Coues white-tailed and mule deer from the southwestern United States (Personal experience, personal communication C. Dunn- Yavapai College, D. King- King Guide Service)

Weathering class	Weathered antler characteristics
1	Protected side (side of antler lying against ground) and exposed sides of antler brown in color, no cracks visible on antler.
2	Protected side of antler brown, exposed side of antler faded brown, no longitudinal cracks visible.
3	Protected side of antler faded brown, exposed side of antler white with small longitudinal cracks visible.
4	Protected side of antler white with small longitudinal cracks, exposed side of antler white with larger and longer longitudinal cracks.
5	Protected side of antler white in color with larger and longer cracks > 3 cm long, exposed side white longer and larger cracks > 5 cm long.
6	Protected side of antler white in color, cracks > 5cm long. Exposed side of antler white in color cracks > 8 cm long with surface of antler beginning to exfoliate. Tips of tines deteriorated.
7	Protected and exposed side of antler white in color with pinkish stain. Surface of antler beginning to exfoliate. Large cracks along the entire length of main beam. Tips of tines completely deteriorated. Base of tines deteriorated. Crumbly to the touch, main beam deteriorated, only larger portions remain.