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Genetic diversity and structure among subspecies of white-tailed deer in Mexico

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The white-tailed deer (*Odocoileus virginianus*) occurs over a broad latitudinal range from South America to Canada. Thirty-eight subspecies are recognized, 14 of which occur in Mexico. Genetic studies in Latin America are lacking and the diversity and structure of white-tailed deer in Mexico are unknown. We sampled white-tailed deer from 13 sites in the range of 5 subspecies occurring in Mexico, *O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, *O. v. sinaloae*, and *O. v. yucatanensis*. We estimated genetic diversity and structure based on 12 microsatellite DNA loci. Observed heterozygosity (H_O) was comparable to that of white-tailed deer in the United States (H_O = 0.53–0.64), with the exception of *O. v. yucatanensis* (H_O = 0.41). We observed statistically significant genetic structure among all 13 sites (F_{ST} = 0.15). Analysis of molecular variance revealed that grouping sites by subspecies (F_{SC} = 0.09) or geographic region (F_{SC} = 0.13–0.14) explained a moderate portion of genetic variation. However, no higher-level group minimized differentiation among populations within the subspecies or regional groups (F_{ST} = 0.16–0.20). Pairwise genetic distances among sites were correlated with geographic distance (r^2 = 0.38), but some geographically proximate sites were genetically differentiated (F_{ST} > 0.20), especially in the Yucatan. Deer in the Yucatan were genetically differentiated from other subspecies and had comparatively lower genetic diversity, consistent with the biogeographic history of the region. Populations of white-tailed deer in Mexico are subject to a range of management challenges. Additional research is needed to understand the effect of management on the diversity and genetic structure of white-tailed deer.

Key words: genetic diversity, genetic structure, Mexico, microsatellites, *Odocoileus virginianus*, subspecies, white-tailed deer

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White-tailed deer (*Odocoileus virginianus*) have one of the largest latitudinal distributions of any species of ungulate, ranging from Bolivia and Venezuela in South America to northern Canada (Demarais et al. 2000). White-tailed deer differ in body size, coat color, and other physical features throughout this range and 38 subspecies are recognized (Baker 1984). Twenty subspecies of white-tailed deer occur exclusively in Latin American countries, where their conservation status is often uncertain (Weber and González 2003). In Mexico, 14 subspecies are recognized and white-tailed deer occur in every Mexican state except Baja California.

Subspecies of white-tailed deer were originally defined on the basis of discontinuities in the geographical distribution of phenotypic traits and minor morphology (Hall 1981). Many of the subspecies' descriptions are an extension of Zimmermann's (1780) original morphological criteria. Hall (1981) proposed a map of subspecies distribution throughout the species' range that is still widely used, but may not reflect current distribution after several centuries of European exploitation and management. Few studies have evaluated genetic differentiation among subspecies of white-tailed deer, and genetic studies in Latin America are very poorly represented in the published

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literature. Previous studies of white-tailed deer have revealed that the number of unique genetic stocks is often fewer than the number of recognized subspecies (DeYoung et al. 2003b; Honeycutt 2000; Moscarella et al. 2003).

In northern states of Mexico, white-tailed deer are an important part of the regional economy through revenues derived from sport hunting, especially for the geographically widespread subspecies *O. v. texanus* and for the geographically and morphologically distinctive subspecies *O. v. couesi*. Elsewhere, the conservation status, genetic diversity, and genetic differentiation within and among subspecies are poorly understood. After an exhaustive analysis of ecological and morphological data, Mandujano et al. (2010) used an ecoregional classification to group the 14 Mexican subspecies in 3 ecogroups or management units, Ecoregion I in northeastern Mexico, composed of *O. v. texanus*, *O. v. miquihuanensis*, and *O. v. carminis*; Ecoregion II in the Pacific and central region of Mexico, including *O. v. couesi*, *O. v. mexicanus*, *O. v. sinaloae*, *O. v. oaxacensis*, and *O. v. acapulcensis*; and Ecoregion III in the Gulf of Mexico and southern region of the country, including *O. v. veraecrucis*, *O. v. thomasi*, *O. v. toltecus*, *O. v. nelsoni*, *O. v. truei*, and *O. v. yucatanensis*.

A genetic evaluation of population structure and diversity within and among subspecies based in ecoregions and subspecies classification is clearly needed to inform conservation and management priorities in Mexico. For instance, subspecies representing unique genetic stocks could warrant increased conservation attention to maintain genetic integrity (Ryder 1986). Some regions of the country are experiencing increasing urbanization or illegal harvest and unique stocks could be at risk. In other regions, intensive management of deer for hunting involves transplants, and movement of individuals across subspecies boundaries could dilute unique stocks. Furthermore, examination of genetic data can reveal aspects of the historical biogeography of white-tailed deer. The overall objective of this study was to gather genetic data from several subspecies of deer in Mexico. Specific objectives were to assess and compare genetic diversity among the subspecies; and to evaluate the validity of subspecies classification for the delineation of regional population groupings.

Materials and Methods

Study area.—We obtained 90 samples (76 males and 14 females) at 13 sites throughout the range of 5 subspecies: *O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, *O. v. sinaloae*, and *O. v. yucatanensis* (Fig. 1). Samples were collected from conservation, management, and sustainable utilization units, and consisted of hair, skin, or muscle biopsies from harvested deer; cast antlers; or fecal samples. Animals were captured and handled following guidelines approved by the American Society of Mammalogists (Sikes et al. 2011). Sampling was concentrated at 4 geographic regions of the country, Northeast (*O. v. texanus* and *O. v. carminis*), Central-East (*O. v. veraecrucis*), Central-West (*O. v. sinaloae*), and Southeastern (*O. v. yucatanensis* [Fig. 1; Appendix I]).

DNA extraction and amplification.—We extracted DNA from fecal samples using the ZR Fecal DNA commercial kit designed for feces (Zymo Research, Orange, California). We extracted DNA from hair, skin, muscle, and antler tissues using commercial kits (Genelute Mammalian Genomic DNA [Sigma, St. Louis, Missouri] or Puregene Tissue Core kit B [Gentra Systems, Minneapolis, Minnesota]). We used microsatellite markers because they are highly polymorphic and appropriated for population genetic structure (Selkoe and Toonen 2006), so we amplified 12 microsatellite DNA loci using the polymerase chain reaction, with primers designed for species of ungulates, including *Bos taurus*, *Ovis aries*, *O. virginianus*, and *Odocoileus hemionus* (Appendix II). Each forward primer was labeled with a fluorescent tag. Heterologous polymerase chain reaction primers have been broadly used in species of ungulates to eliminate the need to design species-specific loci (Engel et al. 1996). We used reagent concentrations reported in previous studies (Anderson et al. 2002; DeYoung et al. 2003a), and designed a set of thermal cycler conditions to enable efficient amplification (Appendix II). We mixed the polymerase chain reaction products with an internal size standard (GeneScan 500 [ROX]; Applied Biosystems, Foster City, California) and a denaturing mixture of formamide (Hi-Di; Applied Biosystems). We loaded the polymerase chain reaction product mixtures onto an ABI 3130 Genetic Analyzer (Applied Biosystems) for separation and detection. We binned and assigned alleles using GeneMapper (Applied Biosystems) software, followed by visual inspection and verification.

Genetic diversity and population structure.—We indexed genetic diversity at the subspecies level by estimating allelic diversity (mean number of alleles per locus [A]) and observed and expected heterozygosity (H_O and H_E, respectively) using the software GENETIX 4.05 (Belkhir et al. 1996). Genetic diversity estimates are not directly comparable if the number of individuals in each sample differs because a larger sample is more likely to include rare alleles. We calculated allelic richness (A_R), a rarefaction approach that adjusts the diversity estimate for differences in sample size, using the computer program HP-RARE (Kalinowski 2005).

We screened loci for null alleles using the computer program MICRO-CHECKER (Van Oosterhout et al. 2004); in the case of occurrence of null alleles, for each sampling site allele frequencies in the presence of null alleles were estimated using the computer program FreeNA (Chapuis and Estoup 2007). We evaluated departure from Hardy–Weinberg proportions using an exact test implemented in the computer program GENEPOP (Raymond and Rousset 1995).

As a basic index of differentiation among subspecies, we identified private alleles using the computer program CERVUS (Kalinowski et al. 2007). We also estimated allelic richness for private alleles (pA_R) using HP-RARE. Next, we evaluated genetic structure and partitioning of genetic variation within and among subspecies using an analysis of molecular variance (AMOVA—Excoffier et al. 1992; Weir 1996; Weir and Cockerham 1984) implemented in the computer program Arlequin 3.1 (Excoffier et al. 2005). We evaluated different

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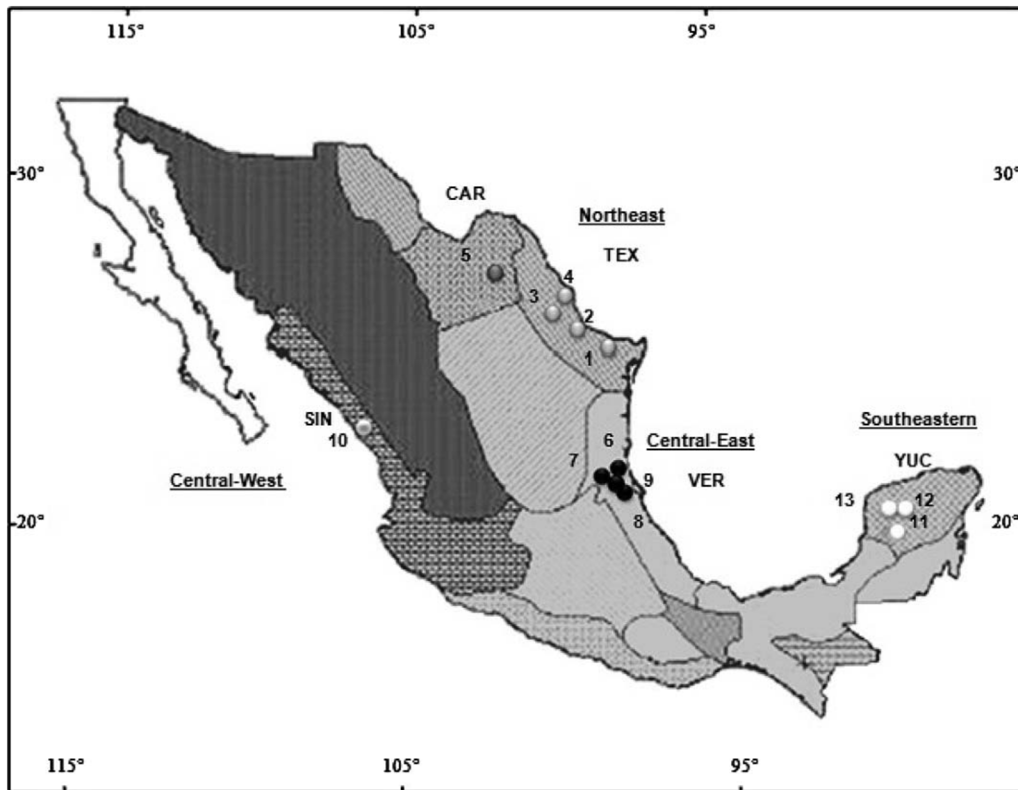

Fig. 1.—Geographic distribution of subspecies of *Odocoileus virginianus* in Mexico, after Méndez (1984). Subspecies are represented as TEX (*O. v. texanus*), CAR (*O. v. carminis*), VER (*O. v. veraecrucis*), SIN (*O. v. sinaloae*), and YUC (*O. v. yucatanensis*). Also presented are the sampling geographic regions: Northeast (TEX 1–4 and CAR 5), Central-East (VER 6–9), Central-West (SIN 10), and Southeastern (YUC 11–13).

hierarchical groupings (models 1, 2, 3, and 4) to quantify the proportion of genetic variation attributed to among sites, subspecies, and regions. Model 1 consisted of the 13 sampling sites (TEX1, TEX2, TEX3, TEX4, CAR5, VER6, VER7, VER8, VER9, SIN10, YUC11, YUC12, and YUC13) and variation was partitioned into within- and among-site components. Model 2 consisted of the 5 subspecies (*O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, *O. v. sinaloae*, and *O. v. yucatanensis*) according to the geographic delimitation described by Hall (1981) and variation was partitioned into within subspecies, among subspecies, and sites within subspecies. In model 3, we evaluated the ecoregional grouping of subspecies proposed by Mandujano et al. (2010), which consisted of 2 regional aggregations, Ecoregion I, including *O. v. texanus* and *O. v. carminis* from northeastern Mexico, and Ecoregion III, including *O. v. veraecrucis* and *O. v. yucatanensis* from the Gulf of Mexico and southern region of the country. The Pacific and central region (Ecoregion II) was not included in the structure model because few samples of *O. v. sinaloae* were available. We partitioned genetic variation into within and among groups, and sites within group. Model 4, which maximized the geographic distance between subspecies, consisted of 2 regional aggregations of sites from the north-central (*O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, and *O. v. sinaloae*) and southern (*O. v. yucatanensis*) regions of the country. We partitioned genetic variation into within and among groups, and sites within group. Statistical significance of the AMOVA was assessed by 10,000 permutations of genotypes among populations or genotypes among subspecies.

We used heterologous primer sets and sampled over a wide geographic distribution, so it is possible that null alleles were present at some or all loci. The presence of null alleles at high frequencies could introduce an upward bias into estimates of genetic structure and differentiation (Chapuis and Estoup 2007). To mitigate for this, we performed additional analyses to investigate relationships among sites and subspecies in the presence of null alleles. First, we estimated pairwise F_{ST} values between subspecies excluding null alleles (ENA; $F_{ST}{}^{(ENA)}$) to account for any bias induced by the presence of null alleles (Chapuis and Estoup 2007). We estimated $F_{ST}{}^{(ENA)}$ using the computer program FreeNA (Chapuis and Estoup 2007). The mean statistics for sampling sites and subspecies were generated by 10,000 replicas over loci, with 95% confidence intervals. Next, we evaluated relationships among sites and subspecies using the chord distance (D_C) of Cavalli-Sforza and Edwards (1967). The D_C is efficient in recovering the true topology of trees constructed from allele frequency data and does not rely on an underlying evolutionary model (Nei and Kumar 2000; Nei et al. 1983). We did not employ a correction for null alleles in the D_C estimate, because simulations have revealed that the correction results in an overestimate of genetic divergence (Chapuis and Estoup 2007). We constructed a neighbor-joining tree (Saitou and Nei 1987) based on D_C to visualize relationships among sites and subspecies. We

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TABLE 1.—Sample size (*N*), mean number of alleles per locus (*A*), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, F_{IS}, and number of loci deviating from Hardy–Weinberg proportions (Hardy–Weinberg deviations; $P < 0.05$), based on data from 12 microsatellite loci in 5 subspecies of *Odocoileus virginianus* sampled in Mexico during 2002–2009.

Subspecies	N	A	$A_{R(6)}$	H_E	H_O	F_{IS}	Hardy–Weinberg deviation ($P < 0.05$)
O. v. texanus	39	11.9	4.3	0.85	0.53	0.38	12
O. v. carminis	12	7.2	3.8	0.78	0.64	0.19	7
O. v. veraecrucis	20	7.8	3.7	0.76	0.59	0.23	7
O. v. sinaloae	3	3.1	3.1	0.64	0.61	0.06	No markers[a]
O. v. yucatanensis	16	5.5	3.2	0.68	0.41	0.42	10

[a] Hardy–Weinberg proportions were calculated in 10 loci because of reduced sampled size ($N = 3$).

assessed statistical support for branches by bootstrapping over loci (1,000 repetitions). We constructed the tree using the computer program PHYLIP version 3.69 (Felsenstein 2005) and viewed the results using the computer program TREEVIEW (Page 1996).

Finally, our sampling sites spanned a broad geographic distribution. Therefore, genetic differentiation may be correlated with geographic distance, often referred to as isolation by distance (Wright 1943). We estimated correlation between genetic and geographic distances, where the pairwise $F_{ST}^{(ENA)}$ values were considered dependent variables and geographic distance between sites was the independent variable. Geographic distances (km) were estimated using the Instituto Nacional de Estadística Geografía e Informática (Statistics, Geography, and Informatics National Institute) distance measurement tool of Mexico Digital Map, version 5.0 (Instituto Nacional de Estadística Geografía e Informática 2007). A regression analysis between $F_{ST}^{(ENA)}/(1 - F_{ST}^{(ENA)})$ and geographic distances of 13 sampling sites was performed using GENEPOP software. Also, a statistical significance test was calculated according to Mantel (1967) using 10,000 permutations. Regression analysis was performed using STATISTICA version 8.0 software (StatSoft, Inc. 2007).

RESULTS

The microsatellite loci were variable in the Mexican deer, with average number of alleles per locus ranging from 5.5 to 11.9. (Table 1). *O. v. texanus* had qualitatively higher levels of genetic diversity, whereas *O. v. yucatanensis* had lower diversity; the low number of samples from *O. v. sinaloae* prevented any robust comparisons. Values of H_O were moderate (0.41–0.64) and lower than those of H_E ($F_{IS} > 0$). F_{IS} ranged from 0.06 to 0.42 and differed from 0 in *O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, and *O. v. yucatanensis*. Many of the loci departed from Hardy–Weinberg equilibrium at the regional (subspecies) level. The OCAM locus displayed a consistently large deficit of heterozygotes, and no amplifiable alleles were detected in the 20 samples of *O. v. veraecrucis*. We discarded this locus in all subsequent analyses.

We observed private alleles in all subspecies. Overall, *O. v. texanus* and *O. v. veraecrucis* displayed the greatest number of private alleles, and even the smallest sample of *O. v. sinaloae* contained 2 private alleles (Appendix III). Private alleles were present at all loci, where BM4208 and BM203 contained both the greatest total number of alleles (22 and 27, respectively) and private alleles (14 and 12, respectively). When sampling size was adjusted to 6 genes, pA_R varied between 4.3 alleles in *O. v. texanus* and 3.1 alleles in *O. v. sinaloae*. A_R was calculated on 11 microsatellites; OCAM was deleted in this analysis.

We observed moderate levels of genetic structure in all hypothesized AMOVA groupings. The lowest F_{ST} value ($F_{ST} = 0.15$) was observed among sites, with no additional hierarchical levels (Table 2). Grouping sites by subspecies ($F_{ST} = 0.17$) or ecoregions ($F_{ST} = 0.16$–0.20) resulted in increased genetic structure. The among-group components of variance for hierarchical AMOVA models explained 2.4–8.7% of the variation. In contrast, among-site components of variance explained 8.6–13.3% of variation. Pairwise $F_{ST}^{(ENA)}$ values among all sites revealed significant structure among sites within subspecies (Appendix IV), especially for *O. v. yucatanensis* and *O. v. veraecrucis*, where $F_{ST}^{(ENA)}$ values > 0.20 and > 0.05, respectively, occurred among sites separated by less than 100 km (Appendix V). Despite the structure within regions, sites clustered together according to subspecies in the neighbor-joining tree (Fig. 2). Tree branches for the subspecies *O. v. carminis* received moderate bootstrap support, whereas the groups *O. v. yucatanensis* and *O. v. veraecrucis* were strongly supported.

Overall, genetic differentiation among subspecies was associated with geographic distance ($r^2 = 0.38$, $F_{1,76} = 46.21$, $P < 0.01$; Appendix V). The greatest genetic distances occurred in comparisons between *O. v. yucatanensis* and other subspecies groups. We also observed a trend for large genetic distances between *O. v. sinaloae* and other subspecies (Appendix IV). The small sample of *O. v. sinaloae* precluded rigorous interpretation, but the observed genetic differentiation would be consistent with the geographic distance between sites.

DISCUSSION

We observed departures from Hardy–Weinberg equilibrium at many loci, in all cases heterozygote deficits. If the loci are not influenced by selection, Hardy–Weinberg disequilibrium may be caused by population substructure (i.e., Wahlund effect—Hedrick 2000) or the presence of null alleles at high frequencies. Overall, about one-half of the loci had null allele frequency estimates exceeding 20% when they were analyzed by pooling sampling sites by subspecies. However, when null alleles were examined independently in each sampling site, 0, 1, 2, or 3 loci were observed with null alleles. Five loci with null alleles were observed exclusively in *O. v. yucatanensis* (YUC11). The frequency of null alleles in congeneric species tends to increase with increasing phylogenetic distance from the focal species for which the locus was derived (Li et al. 2003); all but 2 loci used in this study were originally discovered in sheep and cattle. Null alleles have been observed in previous evaluations of bovine and

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TABLE 2.—Analysis of molecular variance for hierarchical population groups of white-tailed deer (*Odocoileus virginianus*) in Mexico, representing 5 subspecies (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Model	Source of variation	*d.f.*	Sum of squares	Variance components	% of variation	Structure	*P*
Global over 13 sites[a]	Among populations	12	133.31	0.58	15.1	F_{ST}: 0.15	***
	Within populations	167	541.99	3.25	84.9		
	Total	179	675.31	3.82			
Subspecies[b]	Among groups	4	71.89	0.31	8.0	F_{SC}: 0.09	***
	Among populations within groups	8	61.42	0.34	8.6	F_{ST}: 0.17	***
	Within populations	167	541.99	3.25	83.4	F_{CT}: 0.08	***
	Total	179	675.31	3.89			
2 groups[c]	Among groups	1	19.12	0.09	2.4	F_{SC}: 0.14	***
	Among populations within groups	10	104.92	0.51	13.3	F_{ST}: 0.16	***
	Within populations	162	525.83	3.25	84.3	F_{CT}: 0.02	**
	Total	173	649.86	3.85			
2 groups[d]	Among groups	1	27.91	0.35	8.7	F_{SC}: 0.13	***
	Among populations	11	105.40	0.47	11.4	F_{ST}: 0.20	***
	Within populations	167	541.99	3.25	79.9	F_{CT}: 0.09	**
	Total	179	675.31	4.06			

[a] Sampling sites (TEX1, TEX2, TEX3, TEX4, CAR5, VER6, VER7, VER8, VER9, SIN10, YUC11, YUC12, and YUC13).

[b] Subspecies are *O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, *O. v. sinaloae*, and *O. v. yucatanensis*.

[c] Northeastern (*O. v. texanus* and *O. v. carminis*) and Gulf of Mexico–southern (*O. v. veraecrucis* and *O. v. yucatanensis*).

[d] North-central (*O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, and *O. v. sinaloae*) and southern (*O. v. yucatanensis*).

ovine loci in white-tailed deer (Anderson et al. 2002; DeYoung et al. 2003a), where the inclusion of known parent–offspring pairs verified the presence of nulls.

Null alleles can be estimated in population studies if observed heterozygote deficiencies have no other origin (i.e., Wahlund effect or substructure—Chapuis and Estoup 2007). The presence of substructure among geographically proximate sampling sites cannot be totally discounted, because white-tailed deer exhibit a pattern of female philopatry and male-biased dispersal that results in fine-scale social and genetic structure in some regions (Miller et al. 2010; Purdue et al. 2000). However, pooled microsatellite data from 13 sites sampled across a broad geographic region of the southeastern United States displayed fewer instances of heterozygote deficit than in our study (DeYoung et al. 2003a). Overall, it appeared likely that the heterozygote deficits were partly or wholly due to the presence of null alleles in some loci. Therefore, we used appropriate estimates of allele frequencies and genetic differentiation to mitigate for the effects of null alleles in our analyses (Chapuis and Estoup 2007).

Genetic diversity in the geographic range of *O. v. texanus*, *O. v. carminis*, and *O. v. veraecrucis* was comparable (H_O = 0.53–0.64) to previously reported values for white-tailed deer (H_O = 0.64–0.78) in the southern United States (DeYoung et al. 2003a, 2003b). Different factors, such as rapid population expansion, habitat continuity, multiple paternity, and prolificacy have been described for the retention of variability in white-tailed deer populations (DeYoung et al. 2003a, 2003b). However, we observed less genetic diversity in *O. v. yucatanensis* ($H_O = 0.41$) than in other subspecies. In order to discard the influence of null alleles, sampling sites for *O. v. yucatanensis* were analyzed independently. When loci with high null allele frequency were deleted, $H_O < 0.53$ also was observed in the 3 sampling sites studied (YUC11, $H_O = 0.51$; YUC12, $H_O = 0.50$; YUC13, $H_O = 0.51$).

We observed moderate genetic structure among sites, indicative of genetic differentiation across the broad geographic area sampled. Genetic structuring among sites in Mexico was more pronounced than among white-tailed deer sampled across comparable geographic extent in the southeastern United States (F_{ST} = 0.05–0.08—DeYoung et al. 2003b). Structuring in the Mexican populations may be influenced by distribution, habitat, or other regional geographic features. An AMOVA among sites produced the lowest estimate of genetic structure, whereas the inclusion of subspecies or regional hierarchies increased genetic structure. Most variation (>80%) was contained within populations. The increase in structure due to addition of hierarchical levels was due to structure within the subspecies' range. For instance, we observed relatively high structure among sites separated by geographic distances < 100 km in the range of *O. v. yucatanensis* and moderate structuring within *O. v. veraecrucis* and *O. v. texanus*. We detected private alleles in all subspecies groups, even in the small sample of *O. v. sinaloae*. Private alleles can be a convenient measure of population differentiation (Kalinowski 2004), and the presence of private alleles reinforces the conclusions of the structuring analyses.

Overall, some of the genetic structure can be explained by the geographic distance among sites, as evidenced by the positive correlation between genetic and geographic distance. Nevertheless, the uneven genetic structuring within regions implies a complex pattern of differentiation among sites. We could not perform a detailed genetic evaluation of all subspecies. However, examination of our genetic data on 5 subspecies revealed differentiation within the subspecies as well as among subspecies, regardless of the hypothesized regional groups. For instance, when structure based in the northeastern and Gulf of Mexico–southern ecoregions as such was proposed by Mandujano et al. (2010), the lowest genetic variance was observed. In the comparison between subspecies we observed moderate to high differentiation between *O. v.*

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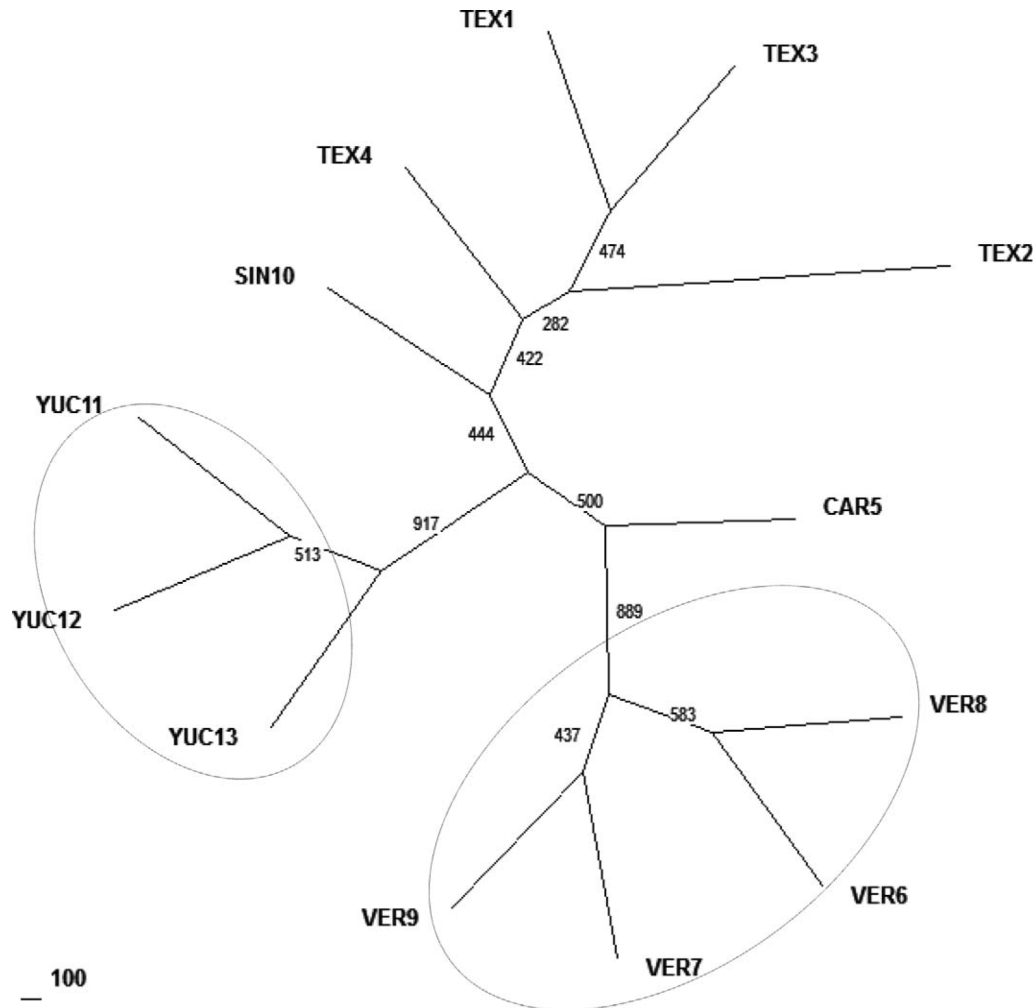

FIG. 2.—Neighbor-joining tree based on chord distance (D_C) from 12 microsatellite DNA loci for 13 sampling sites of white-tailed deer in Mexico representing 5 recognized subspecies. Branch lengths show genetic distance according to scale and numbers at nodes show bootstrap support derived from 1,000 resamplings over loci. Subspecies are represented as TEX (*O. v. texanus*), CAR (*O. v. carminis*), VER (*O. v. veraecrucis*), SIN (*O. v. sinaloae*), and YUC (*O. v. yucatanensis*).

texanus, *O. v. carminis*, and *O. v. veraecrucis* (Northeast–Central-East area), differentiation within the range of *O. v. yucatanensis* (Southeastern area), and a trend for differentiation between all subspecies and *O. v. sinaloae* (Central-West area).

Mandujano et al. (2010) demonstrated that 13 of the 14 subspecies found in Mexico (excluding *O. v. toltecus*) could be organized in northern and southern groups according to total body length, chest height, and antler measurements. The northern subspecies were physically larger, including *O. v. texanus*, *O. v. carminis*, *O. v. miquihuanensis*, *O. v. veraecrucis*, *O. v. mexicanus*, and *O. v. couesi*. The southern subspecies were smaller, including *O. v. sinaloae*, *O. v. thomasi*, *O. v. yucatanensis*, *O. v. truei*, *O. v. oaxacensis*, *O. v. acapulcensis*, and *O. v. nelsoni*. Overall, the north-central and southern regions also maximize the genetic variance between populations and groups and minimize the intrapopulation variance (<80%). Southern subspecies typically occur in tropical environments, whereas the northern subspecies occur in shrublands and temperate forest. Differences in habitat and soil quality among regions can result in significant differences in body mass, skeletal size, and antler size (Strickland and Demarais 2000, 2008). Furthermore, the patterns of genetic structure and differentiation we observed may reflect the historical biogeography of the country. For instance, *O. v. texanus* had the most private alleles and comparatively lower structuring within the subspecies' range, which may reflect robust historical population sizes or greater connectivity among populations. Because *O. v. texanus* has been the principal subspecies managed for trophy hunting, human intervention also could be influencing our results. Although an artificial connectivity between populations of *O. v. texanus* is possible due to human intervention, deer habitat is continuously distributed in the region of the subspecies *O. v. texanus*.

Elsewhere, changes in climate at the end of the Pleistocene resulted in patchy distribution of white-tailed deer (Ellsworth et al. 1994). For instance, suitable habitat in the range of *O. v. carminis* and *O. v. couesi* contracted to isolated upper-elevation sites with adequate rainfall to sustain deer year-round as the

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climate became drier (Heffelfinger 2006). We were unable to test hypotheses relating to structuring within the range of *O. v. carminis*, because samples were available from only 1 site, whereas no samples were available from *O. v. couesi*. However, samples of *O. v. carminis* displayed similar levels of genetic differentiation from both the geographically proximate *O. v. texanus* and the more distant *O. v. veraecrucis*. A recent study of *O. hemionus* also found a complicated pattern of recolonization for subspecies of mule deer and black-tailed deer after the Last Glacial Maximum (Latch et al. 2009).

We observed comparatively low diversity and pronounced structuring within the Yucatan relative to other areas of Mexico. Furthermore, all subspecies groups displayed significant genetic differentiation from the Yucatan samples. The biogeographic history of the Yucatan region may have resulted in isolation of deer populations from the mainland during sea-level rises between Pleistocene glaciations, as hypothesized for white-tailed deer in Florida (Ellsworth et al. 1994). In bats, a recent phylogeographic study conducted on Davy's naked-backed bat (*Pteronotus davyi*) supports the existence of 2 distinct genetic lineages (Guevara-Chumacero et al. 2010). The Pacific–Gulf Coast lineage and Southeast lineage discussed in that study are distributed according to 2 refuges (Arc and Soconusco refuges) located in southern Mexico during the Pleistocene (Guevara-Chumacero et al. 2010). A complementary analysis based on mitochondrial DNA is required to deepen understanding of the genetic differences found in *O. virginianus* from north-central and southern regions of Mexico. Alternatively, populations on the periphery of a species' range often display less diversity than central populations because of uneven exchange of dispersers and smaller effective population size (Schwartz et al. 2003). The Yucatan also has a long history of human occupation, as evidenced by the rich assortment of archaeological sites in the region. White-tailed deer were undoubtedly exploited by Native Americans; the degree to which indigenous peoples may have affected deer population genetic attributes is unknown (Emery 2007). However, we cannot rule out the effects of contemporary processes on genetic diversity and structure in the Yucatan region. Regardless, further study is warranted to better quantify genetic diversity in the Yucatan region and to understand the causal factors for reduced diversity.

Our analysis provides a preliminary investigation of genetic diversity and structure of white-tailed deer in Mexico and lays an important foundation for further research. Overall, the white-tailed deer we sampled had levels of neutral genetic diversity that were comparable to those of white-tailed deer in other regions, with the sole exception being sites in the Yucatan. Genetic differentiation may occur within subspecies boundaries, but it is not clear if differentiation is the result of contemporary or historical factors. Regardless, the comparatively lower genetic variation and pronounced genetic structuring we observed over modest geographic distances in the Yucatan region were not present elsewhere in our sample of deer from Mexico. Similarly, white-tailed deer remain an important part of the local diet in parts of central and southern Mexico, where deer are subject to illegal hunting activity (Weber and González 2003). The conservation status of white-tails is poorly known and more data are needed to ensure the sustainable use of deer in the southern geographic regions.

In other regions of Mexico, changes to management may be warranted even though white-tailed deer are thriving. For instance, in the north-central area of Mexico, the subspecies *O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, and *O. v. couesi* are valued for sport hunting (Mandujano et al. 2010). These 4 subspecies are recognized with separate trophy categories designated by Safari Club International or the Boone and Crockett Club. However, white-tailed deer are included in the list of species of diversified livestock in Mexico (Villarreal 1999). Therefore, some populations are subject to management strategies such as translocation across subspecies boundaries. Translocations and other intensive management should be conducted with care to ensure that potentially unique lineages are preserved until the biogeographic history of white-tailed deer is better understood.

Resumen

El venado de cola blanca (*Odocoileus virginianus*) se distribuye en un amplio rango latitudinal desde el sur de América hasta Canadá. De las 38 subespecies reconocidas, 14 habitan en México. Debido al número limitado de estudios genéticos en Latinoamérica, la diversidad y estructura poblacional del venado de cola blanca en México es desconocida. En este estudio, muestreamos venado de cola blanca de 13 sitios ubicados dentro del rango de distribución de 5 subespecies de México, *O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, *O. v. sinaloae*, and *O. v. yucatanensis*. La diversidad y estructura genética fue estimada con 12 marcadores microsatélites. La heterocigosidad observada fue comparable a lo observado en el venado de cola blanca de Norteamérica (H_O = 0.53–0.64), con excepción de *O. v. yucatanensis* ($H_O = 0.41$). Se observó una estructura genética significativa entre los 13 sitios de muestreo ($F_{ST} = 0.15$). El análisis de varianza molecular reveló que los sitios de muestreo agrupados por subespecie ($F_{SC} = 0.09$) o región geográfica (F_{SC} = 0.13–0.14) explicaron una porción moderada de la variación genética. Sin embargo, la diferenciación entre las poblaciones no fue minimizada a un nivel de agrupamiento mayor, es decir dentro de subespecies o grupos regionales (F_{ST} = 0.16–0.20). La distancia genética entre sitios de muestreo estuvo correlacionada con la distancia geográfica ($r^2 = 0.38$), pero algunos sitios geográficamente próximos estuvieron genéticamente diferenciados ($F_{ST} > 0.20$), especialmente en Yucatán. El venado de cola blanca de Yucatán fue diferenciado genéticamente de las otras subespecies y fue comparativamente el de menor diversidad genética, lo cual es consistente con la historia biogeográfica de la región. Las poblaciones de venado cola blanca en México están sujetas a diversos retos de manejo. Por lo tanto, se requiere de investigación adicional para comprender el efecto sobre su diversidad y estructura genética.

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APPENDIX I

Sampling sites, number of samples (*N*), subspecies, and geographic coordinates for subspecies of *Odocoileus virginianus* sampled in Mexico during 2002–2009.

Sampling site	*N*	Subspecies	Geographic location	
TEX1	10	*O. v. texanus*	Reynosa, Tamaulipas	26°04′16″N, 98°17′51″W
TEX2	9	*O. v. texanus*	Guerrero, Tamaulipas	26°43′34″N, 98°28′36″W
TEX3	10	*O. v. texanus*	Anáhuac, Nuevo León	27°13′58″N, 100°08′04″W
TEX4	10	*O. v. texanus*	Nuevo Laredo, Tamaulipas	27°19′60″N, 99°32′60″W
CAR5	12	*O. v. carminis*	Muzquiz, Coahuila	27°54′30″N, 101°34′23″W
VER6	3	*O. v. veraecrucis*	Ébano, San Luis Potosí	22°12′53″N, 98°22′51″W
VER7	9	*O. v. veraecrucis*	Tamuín, San Luis Potosí	22°07′20″N, 98°37′57″W
VER8	5	*O. v. veraecrucis*	Pánuco, Veracruz	21°53′07″N, 98°20′28″W
VER9	3	*O. v. veraecrucis*	Pánuco, Veracruz	21°57′34″N, 98°24′170″W
SIN10	3	*O. v. sinaloae*	Mazatlán, Sinaloa	23°14′01″N, 106°25′45″W
YUC11	5	*O. v. yucatanensis*	Tzucacab, Yucatán	20°04′18″N, 89°02′59″W
YUC12	8	*O. v. yucatanensis*	Mérida, Yucatán	20°58′47″N, 89°36′57″W
YUC13	3	*O. v. yucatanensis*	Mérida, Yucatán	20°51′39″N, 89°36′46″W

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Appendix II

Microsatellite loci, primer sequences, and amplification profiles for 12 DNA microsatellites amplified in white-tailed deer (*Odocoileus virginianus*). F = forward; R = reverse.

Locus	Primer sequences	Profile[a]	Reference
BM203	F: GGGTGTGACATTTTGTTCCC R: CTGCTCGCCACTAGTCCTTC	TD55	Bishop et al. 1994
BM4208	F: TCAGTACACTGGCCACCATG R: CACTGCATGCTTTTCCAAAC	TD55	Bishop et al. 1994
D15	F: AAAGTGACACAACAGCTTCTCCAG R: AACGAGTGTCCTAGTTTGGCTGTG	TD55	Moore et al. 1994
MAF 36	F: ATATACCTGGGAGGAATGCATTACG R: TTGCAAAAGTTGGACACAATTGAGC	TD55	Arranz et al. 2001
TGLA126	F: CTAATTTAGAATGAGAGAGGCTTCT R: TTGGTCTCTATTCTCTGAATATTCC	TDTG	Georges and Massey 1992
MAF 70	F: CACGGAGTCACAAAGAGTCAGACC R: GCAGGACTCTACGGGGCCTTTGC	TD60	Arranz et al. 2001
MSTN01	F: ATAAAGTCCGTTGGTTTACG R: CGACTTGTGAATCTTACTGA	TD55	De la Rosa-Reyna et al. 2006
D	F: AGAGCCTCGTCTTTCATTC R: TTGCTGCTTGCTTGTCTAAT	TD55	Jones et al. 2000
Cervid1	F: AAATGACAACCCGCTCCAGTATC R: TCCGTGCATCTCAACATGAGTTAG	TD55	DeWoody et al. 1995
BM848	F: TGGTTGGAAGGAAACTTGG R: CCTCTGCTCCTCAAGACAC	TD55	Bishop et al. 1994
BM6438	F: TTGAGCACAGACACAGACTGG R: ACTGAATGCCTCCTTTGTGC	TD55	Bishop et al. 1994
OCAM	F: CCTGACTATAATGTACAGATCCCTC R: GCAGAATGACTAGGAAGGATGGA	TD55	Moore et al. 1992

[a] Touchdown polymerase chain reaction temperature profiles: TD55: initial denaturation at 95°C for 5 min; 5 cycles of 95°C for 45 s, 62°C for 45 s (decreasing 2°C each cycle), 72°C for 45 s; 30 cycles of 95°C for 45 s, 55°C for 45 s, 72°C for 45 s; final extension at 72°C for 10 min. TDTG: initial denaturation at 95°C for 5 min; 5 cycles of 95°C for 45 s, 58°C for 45 s (decreasing 2°C each cycle), and 72°C for 45 s; 30 cycles of 95°C for 45 s, 50°C for 45 s, and 72°C for 45 s; final extension at 72°C for 10 min. TD60: initial denaturation at 95°C for 5 min; 5 cycles of 95°C for 45 s, 65°C for 45 s (decreasing 2°C each cycle), 72°C for 45 s; 30 cycles of 95°C for 45 s, 60°C for 45 s, 72°C for 45 s; final extension at 72°C for 10 min.

Appendix III

Number of alleles, size range, identity of private alleles, and allelic richness of private alleles ($pA_{R(6)}$) detected in 12 microsatellite DNA loci amplified in 5 subspecies of white-tailed deer (*Odocoileus virginianus*) sampled in Mexico. bp = base pairs.

			Private alleles				
Locus	Total no. alleles	Size range (bp)	*O. v. texanus*	*O. v. carminis*	*O. v. veraecrucis*	*O. v. sinaloae*	*O. v. yucatanensis*
BM203	27	194–302	194, 196, 202, 228, 230	260, 302	198, 264, 266	238	244
BM4208	22	134–178	136, 138, 142, 144, 148, 160, 162, 166, 170	—	134, 150, 178	—	146, 164
D15	12	224–248	226, 228, 232	—	—	—	—
MAF36	10	102–122	108, 114	—	—	—	—
TGLA126	10	100–124	100, 124	—	118	—	116
MAF70	19	100–134	100	96	—	—	—
MSTN01	8	121–135	121, 123	—	135	—	—
D	15	150–190	150, 190	182	156, 174	—	—
CERVID1	16	160–194	194	—	—	—	—
BM848	14	364–390	—	372	386	—	—
BM6438	15	252–280	258, 280	260	—	—	266
OCAM	12	194–222	196, 222	—	—	194	—
Total	180		31	6	11	2	5
$pA_{R(6)}$			1.25	0.80	0.89	0.86	0.94

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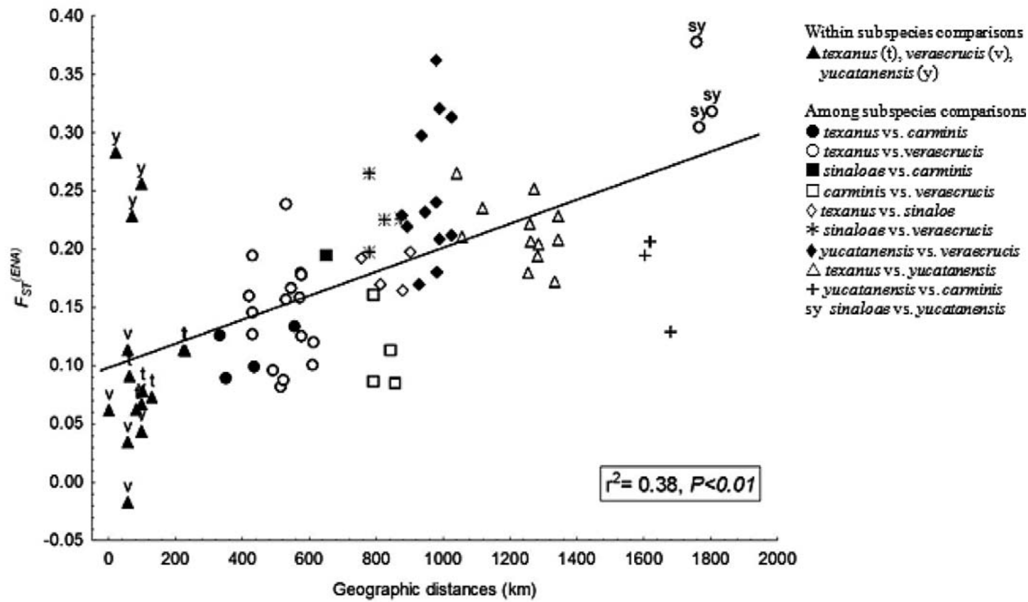
Appendix IV

Genetic distances (D_C) between subspecies of *Odocoileus virginianus* calculated with the Cavalli-Sforza method (below diagonal [Cavalli-Sforza and Edwards 1967]) and $F_{ST}^{(ENA)}$ values (above diagonal).

	Northeast		Central-East	Southeastern	Central-West
	O. v. texanus	*O. v. carminis*	*O. v. veraecrucis*	*O. v. yucatanensis*	*O. v. sinaloae*
O. v. texanus	—	0.08	0.09	0.11	0.15
O. v. carminis	0.12	—	0.09	0.10	0.19
O. v. veraecrucis	0.12	0.10	—	0.17	0.20
O. v. yucatanensis	0.17	0.15	0.19	—	0.26
O. v. sinaloae	0.18	0.19	0.19	0.24	—

Appendix V

Linear relation between pairwise $F_{ST}^{(ENA)}$ values and geographic distance.



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