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Cover Photograph: A White-tailed Deer (*Odocoileus virginianus*) on the windward side of the Naval Station Guantanamo Bay, Cuba. Photograph © Roger W. Portell.

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Insights into the Genetic Origins of White-tailed Deer on the Naval Station Guantanamo Bay

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Abstract - The origin of *Odocoileus virginianus* (White-tailed Deer) on the Naval Station Guantanamo Bay (NSGB) is uncertain. Published reports suggest that White-tailed Deer were introduced into Cuba in the 1850s from North America, with unpublished reports that deer were brought from the United States to NSGB in the early–mid-20th century for hunting. We investigated the genetic origin of deer on NSGB by comparing patterns of variation at 2 regions of the mitochondrial genome and for 8 nuclear microsatellite loci. We compared populations at NSGB to samples from North, Central, and South America. These data suggest that deer found within NSGB did not originate from either the United States or Mexico, but have closer affinities with northern South America. NSGB deer may have arrived from South America naturally via the Lesser Antilles or could have been introduced directly by humans. Future inclusion of data from populations on other Caribbean islands and free-ranging deer from Cuba would provide insight into the geographic origin and modes of colonization of NSGB deer and aid in their management.

Introduction

Odocoileus virginianus (Zimmerman) (White-tailed Deer) are native to the Americas, distributed from southern Canada throughout most of the United States and southward into northern South America. With the exception of desert and mountainous areas of the western United States and the Baja Peninsula, deer are typically abundant throughout their range (Heffelfinger 2011, Smith 1991). White-tailed Deer also occur on many islands in the Caribbean, including Puerto Rico, Jamaica, the US Virgin Islands, and Cuba. On some of these islands, such as Puerto Rico and the Virgin Islands, past introductions are well documented (Heffelfinger 2011). But on other islands, knowledge of how and from where deer arrived is incomplete. On most of these islands, the deer are considered more of

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an agricultural pest than an invasive species that negatively impacts native species (Keehner et al. 2016).

In Cuba, White-tailed Deer occur in mountainous and forested regions throughout the country and some of the outlying islands, including Isla de la Juventud and the Camaguey Archipelago (Borroto-Páez 2009). The Naval Station Guantanamo Bay (NSGB) contains an abundant population of small-sized White-tailed Deer, similar in physical appearance but larger in body size than *O. v. clavium* Barbour and G.M. Allen (Key Deer), the federally protected subspecies of White-tailed Deer found in the nearby Florida Keys (D.L. Reed, pers. observ.).

The long history of human settlement, colonization, and later occupation of Cuba, especially the Guantanamo area, is complex, making the determination of the origin of these deer populations challenging. Deer were reportedly introduced into Cuba from Mexico and/or the southern United States around 1850 (de Vos et al. 1956). Residents of Guantanamo report that deer were brought to the base by US servicemen for hunting between 1930 and 1950, whereas another report states explicitly that the deer were introduced from Florida for hunting around 1954 (Lowney et al. 2005). Although introduction via anthropogenic processes is a likely explanation for deer at NSGB, a plausible alternative hypothesis is that deer naturally colonized Cuba from either the mainland or adjacent islands. The most likely sources for colonization include: (1) Florida, probably the Florida Keys, meaning they could potentially be from the same genetic stock as the federally endangered Key deer; (2) Mexico through the Yucatán Peninsula; or (3) South America via the Lesser and Greater Antilles (Fig. 1).

White-tailed Deer on Cuba appear to be relatively rare and are not legally hunted, but deer found within the fenced boundaries of NSGB were historically much more numerous. In response to overabundance of this deer population and its impact on native vegetation (Areces-Mallea 2010, Sedaghatkish and Roca 1999), deer within NSGB were routinely culled throughout the base starting in 2000, and now their numbers are under control. Knowledge of the geographic origin of the White-tailed Deer populations in NSGB could provide valuable information to guide current and future management strategies, not only at NSGB and on Cuba, but on other islands from the Caribbean. For instance, Key Deer are considered an endangered species under the United States Endangered Species Act and face continual challenges from climate change, limited habitat, and human encroachment. The existence of an additional population of Key Deer derived from historical translocation to NSGB would have ramifications for the management of both populations. Conversely, if one or more of the alternative colonization scenarios were supported, the subspecific and conservation status of the NSGB population would require re-evaluation.

Understanding the origin of the deer within the base also will help lay the ground work for future research on Cuban and Caribbean deer populations, especially should diplomatic relations between Cuba and the United States allow for greater interactions. Here, we use nuclear microsatellites and mitochondrial sequences to investigate whether deer found within NSGB's boundary can be traced to the mainland United States or Mexico, as suggested by previous reports (de Vos et al. 1956), or specifically to Florida as suggested by Lowney et al. (2005). If the deer were introduced from the

United States or Mexico in the 1800s or 1900s, genetic analyses should show a clustering of the NSGB deer with one of those putative source populations.

Field-Site Description

The total area of NSGB, including both land and water, is ~117 km^2 (Fig. 1). NSGB is surrounded on the north, east, and west by a 3 m by 28 km sturdy fence, with the southern boundary bordered by the sea. In addition, there are land mines on both sides of the fence and a biological *Opuntia* fence planted by the Cuban government. The base is divided by the southern portion of Guantanamo Bay and thus has a windward and leeward side. The western, or windward, side of the Bay is mostly hilly, whereas the eastern, or leeward, side is dominated by the flood plain of the Guantanamo River. Land elevations range from 0 (sea-level) to 153 m (steep-sided) in height. Due to limited access and the fact that much of NSGB's land is undisturbed (only 11% of the base is used by the military), much of the native habitat is undisturbed, and native as well as introduced wildlife flourish within the boundaries (Montalvo 2011). The typical habitat of NSGB is tropical desert scrub and dry forest, with many endemic plant and animal species (Lowney et al. 2005).

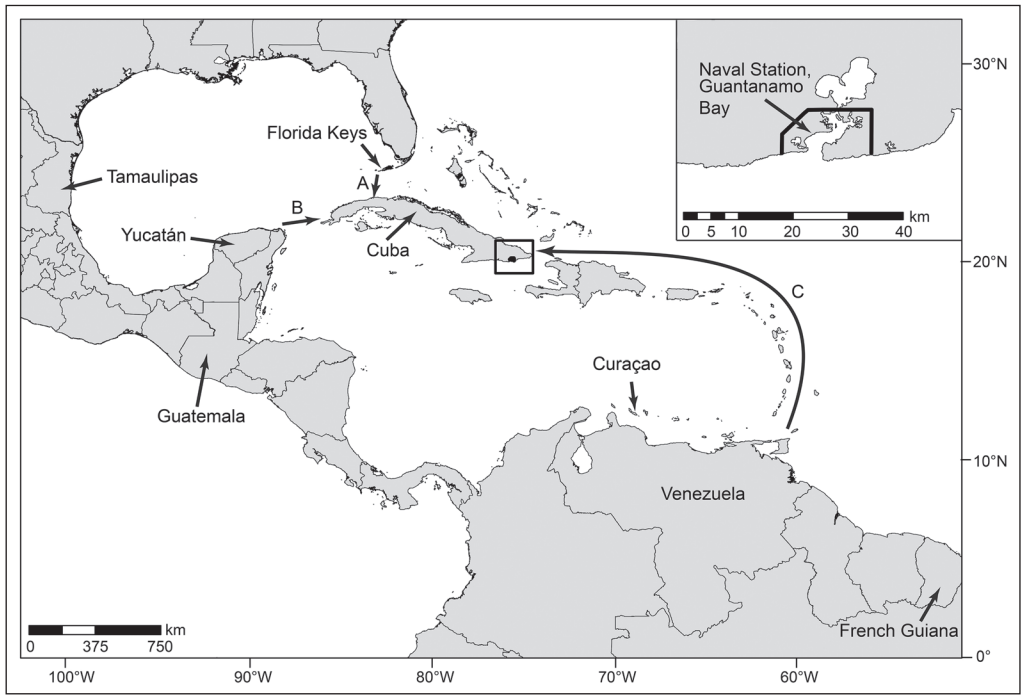

Figure 1. Selected sampling localities of *Odocoileus virginianus* (White-tailed Deer), not including mainland United States. The black box highlights the location of Naval Station Guantanamo Bay (NSGB) in southeastern Cuba; top right inset shows the boundaries of NSGB in greater detail. Arrows with letters show potential colonization routes of White-tailed Deer into Cuba: (A) from the Florida Keys; (B) from Mexico, likely the Yucatán; and (C) from South America up through the Lesser and Greater Antilles.

Deer can be found in both these habitats, with reports of overgrazing by the deer (Areces-Mallea 2010).

Methods

Taxonomic sampling

For the microsatellite analyses, we sampled 135 individuals from the southeastern United States including: 20 Florida Key Deer from Big Pine Key and 115 White-tailed Deer from 14 populations spanning Florida (8 populations, $n = 60$), Alabama (2 populations, $n = 17$), Georgia (2 populations, $n = 18$), and South Carolina (2 populations, $n = 20$). In addition, 5 deer were collected from Curaçao at the Caribbean Marine Biological Institute (CARMABI) Research Station and 20 from NSGB.

We sequenced 2 regions of the mitochondrial genome: cytochrome *b* (cyt *b*) and the control region (also known as D-loop). The cyt *b* dataset ($N = 36$) included samples from NSGB ($n = 20$), Mexico (Tamaulipas [$n = 5$] and Yucatán [$n = 1$]), French Guiana ($n = 1$), and the United States ($n = 5$ including 2 Key Deer from the Florida Keys). Outgroups used in the cyt *b* dataset were *Pudu puda* (Molina) (Southern Pudu), *Hippocamelus antisensis* (d'Orbigny) (Taruca), *Dama mesopotamica* (Brooke) (Persian Fallow Deer), and *Rucervus duvaucelii* (G. Cuvier) (Barasingha).

The control-region dataset ($N = 82$) consisted of White-tailed Deer samples from NSGB ($n = 20$), Mexico (Tamaulipas [$n = 23$] and Yucatán [$n = 3$]), French Guiana ($n = 1$), United States ($n = 9$, including 2 Key Deer from the Florida Keys), Curaçao ($n = 2$), Canada ($n = 1$), Guatemala ($n = 1$), and Venezuela ($n = 19$). Samples used as outgroups were *Cervus elaphus* L. (Red Deer), Southern Pudu, and Taruca. *Odocoileus hemionus* (Rafinesque) (Mule Deer) was excluded from analyses due to known hybridization with White-tailed Deer (Carr and Hughes 1993, Carr et al. 1986). Because lab work was performed in different labs under different conditions, 100% congruence between the datasets was unfortunately not possible. Additionally, more control-region sequences exist on Genbank than do cyt *b* samples, allowing us to have a stronger control-region dataset with more South American samples but not affording us to provide a match with the cyt *b* dataset.

Samples from NSGB were collected in September 2008 and January 2011 during routine culls by station biologists for population management. Sampling of deer was restricted to the leeward side, which spans a large area, because the deployment of mines on the windward side prohibited our collaborator's collecting from this area. The NSGB samples used in both mitochondrial analyses were from the same individuals used in the microsatellite analyses. Samples of deer from the southeastern United States and Mexico were obtained from hunters or road kills. Curaçao samples were collected at CARMABI; amplification of the Curaçao samples was successful for microsatellites and the control region but not for cyt *b*. All sampling followed the guidelines for live animal research published by Sikes et al. (2016).

Florida, Mexico, NSGB, and Curaçao sequences in the mitochondrial datasets are newly generated for this study. The remaining sequences, as well as the outgroup taxa, were obtained from GenBank (see Table S1 in Supplemental File 1,

available online at http://www.eaglehill.us/CANAonline/suppl-files/c173-Mathis-s1). New mitochondrial sequences are deposited in GenBank (accession nos. KX171716–KX171760).

Microsatellite genotyping

The microsatellite analyses represent the combination of data from 2 separate laboratories (those of D.L. Reed and R.L. Honeycutt). However, in both cases the same 8 microsatellite loci originally characterized by Anderson et al. (2002) were used: BM848, BM6506, D, K, N, P, Q, and R. Because these 2 labs used somewhat different procedures to genotype their samples, at least 4 samples of DNA from the southeastern United States and Florida Keys were used to verify concordance in allele scoring from both laboratories. This approach allowed for consistency between the labs and insured that alleles were validated.

Samples from the southeastern United States and the Florida Keys were genotyped and analyzed in the lab of R.L. Honeycutt. DNA was isolated using a phenol/chloroform extraction. Laboratory methods, including the PCR protocol, have been previously detailed in Anderson et al. (2002). Microsatellite genotyping was conducted using forward primers that were labeled with 1 of 3 fluorescent dyes (6-FAM, HEX, or NED), and PCR products were analyzed on an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Allele calls were made using the GeneMapper (Applied Biosystems) software.

Genotyping of the Curaçao and NSGB samples was conducted in the lab of D.L. Reed using the same primers as above, but with modifications to lower the cost of genotyping. Total nuclear DNA from NSGB and Curaçao deer samples were extracted using the Qiagen DNeasy kit for blood and tissue, following the protocol for mammal tissues (Qiagen Inc., Valencia, CA, USA). Fluorescently labeled universal primers M13 (5'-CACGACGTTGTAAAACGAC-3') and CAG (5'-CAGTCGGGCGTCATCA-3') were used, each labeled with a unique fluorescent tag (e.g., FAM, VIC, NED, PET; Applied Biosystems) to co-amplify multiple loci (Blacket et al. 2012). This M13-tag method allows for multiplexing multiple primers to avoid the high expenses of genotyping a single-labeled primer. Each locus was amplified individually in 15-µL multiplex polymerase chain reactions (PCR) containing 7.5 µL of 2X Master Mix (Type-It Microsatellite PCR kit, Qiagen Inc.), 0.2 µL of unlabeled forward primer with tail (10 µM), 0.2 µL of unlabeled reverse primer (10 µM), 0.5 µL labeled universal tail primer (10 µM), 1–2 µL of total genomic DNA (10–20 ng), and sdH_20 added up to final volume. Two thermal cycling profiles were used, both beginning with initial denaturation at 95 °C (5 min) and ending with a final extension of 72 °C (40 min). The touch-down PCR protocol consisted of 10 cycles of 94 °C (30 sec), 60 °C down to 55 °C (-0.5 °C/cycle) for 45 sec, and 72 °C (45 sec) followed by 25 cycles of 94 °C (30 sec), 55 °C (45 sec), and 72 °C (1 min). The PCR products were electrophoresed on 1.5% and 4% agarose gels stained with ethidium bromide, and visualized under ultraviolet light. Dilutions in sdH_20 from amplicons (1:100) were run on an ABI 3730xl 96-capillary sequencer using GeneScan 600 LIZ as an internal size standard (Applied Biosystems) at the

University of Florida Interdisciplinary Center for Biotechnology Research (ICBR; Gainesville, FL, USA). Microsatellite genotypes were scored using GeneMarker v. 1.60 (SoftGenetics, LLC, State College, PA, USA).

Microsatellite genetic analyses

We used the program MSA 4.05 (Dieringer and Schlštterer 2003) to determine number of alleles per locus and allelic richness, Arlequin v. 3.5.1.2 (Excoffier and Lischer 2010) to determine observed and expected heterozygosity (H_O and H_E, respectively), and FSTAT version 2.9.3.2 (Goudet 2001) to obtain mean F_{IS} estimates (an index of the inbreeding of individuals resulting from the non-random union of gametes within a subpopulation; Wright 1969) over all loci per population. We estimated F_{IS} significances after 2720 randomizations and adjusted critical significance levels for multiple comparisons using Bonferroni corrections (Rice 1989). Genotypic disequilibrium among loci was estimated using GENEPOP version 4.2 (Raymond and Rousset 1995; Rousset 2008). For sample sizes exceeding 10 deer per location, we also employed FSTAT 2.9.3.2 (Goudet 2001) to estimate population pairwise distance values of $\Theta_{WC,}$ based on Weir and Cockerham (1984), which is an unbiased, more robust estimator of F_{ST} that can also have negative F_{ST} values.

We inferred population structure with a Bayesian clustering approach implemented in the STRUCTURE software (Pritchard et al. 2000). This method uses individual multi-locus genotypic data to evaluate models assuming different numbers of genetic clusters (K) based on the posterior probabilities given the data and model. Based on the individual multilocus genotypes and the allele frequencies estimated for the reconstructed clusters, each individual's genome was probabilistically partitioned into membership fractions (q; i.e., ancestry) in each cluster. This clustering approach avoids a priori population classification and instead estimates the shared population ancestry of individuals based solely on their genotypes under an assumption of Hardy-Weinberg equilibrium and linkage equilibrium in ancestral populations. All simulations used 50,000 Markov chain Monte Carlo (MCMC) generations in the burn-in phase and 100,000 generations in the data collection phase. We constructed 10 independent runs using default parameters for each K, to ensure equilibration during burn-in and consistency in estimation of the posterior probabilities. Selection of the number of distinct clusters was based on the evaluation of the ΔK statistic (Evanno et al. 2005) and implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2012). We tested 1 through 10 clusters (K). The 10 STRUCTURE runs at each K produced nearly identical individual membership coefficients. We used the run with the highest likelihood of the data given the parameter values for the predominant clustering pattern (i.e., the mode) at each K for plotting with Distruct v1.1 (Rosenburg 2004). We conducted principal coordinates analysis (PCoA) using pairwise genetic difference between individuals calculated in GenAlEx (Peakall and Smouse 2006, 2012) to validate genetic clusters with an independent method. The PCoA is similar to principal components analysis, but is more useful when dealing with discrete characters and when there are fewer individuals than characters (Rohlf 1972).

We calculated matrices of pairwise D_{ps} genetic distances (-ln proportion of alleles shared between locations) with the program MSA 4.05 (Dieringer and Schlötterer 2003), using the allele frequencies at all 8 microsatellite loci. Relationships among populations were reconstructed using the neighbor-joining method (Saitou and Nei 1987) implemented on the matrix of D_{ps} distances. We employed the PHYLIP v 3.6 program package (Felsenstein 2005) to construct the trees, with node support evaluated by bootstrapping across loci (100 replicates).

Phylogenetic analyses of mitochondrial DNA

The mitochondrial DNA of deer from Mexico were amplified and sequenced in the lab of R.W. DeYoung, and deer from Florida, Curaçao, and NSGB were amplified and sequenced in the lab of D.L. Reed. For the Mexican samples, the control region (506 bp) was amplified via PCR with oligonucleotide primers 283 (5'-TACACTGGTCTTGTAAACC-3') and 1115 (5'-ATGACCCTGAAGAARGAACCAG-3'; Bickham et al. 1996). A portion of the cyt *b* gene (1019 bp) was amplified with primers Odh-cytbF-14153 (5'-TCAATGACCAACATCCGAAA-3') and Odh-cytbR-15399 (5'-GGGTGTTGATAGTGGGGCTA-3') (Latch et al. 2009). PCR reactions were in 25-µL volumes containing 12.5 µL Amplitaq Gold PCR Master Mix (Applied Biosystems), 10 pmol of each primer, and 10–50 ng DNA. Reaction conditions consisted of an initial denaturation at 94 °C (12 min) followed by 32 cycles of 94 °C (50 sec), 61 °C (60 sec), 72 °C (2 min), with a final extension at 72 °C for 30 min. PCR products were electrophoresed on 1% agarose gels containing ethidium bromide and viewed under ultraviolet light to verify successful amplification. PCR products were purified using an enzymatic method (ExoSAP-IT; USB Corporation, Wilmington, MD, USA) and cycle sequenced using the BigDye Terminator Cycle Sequencing kit v1.1 (Applied Biosystems). Unincorporated dye terminators were removed using a DyeEx 2.0 spin kit (Qiagen), and each sample was sequenced in both directions on an ABI 3130xl automated DNA sequencer (Applied Biosystems).

For the remaining deer samples from Florida, Curaçao, and NSGB, a portion of the cyt *b* gene (1019 bp) was amplified via PCR using primers MVZ05 (Smith and Patton 1993) and H15915 (Irwin et al. 1991). The control region (506 bp) was amplified with external primers CST 2 and CST 39 (Polziehn et al. 1995), as well as external primers AL3237 and AL3238 and internal primers AL3448 and AL3453 (Moscarella et al. 2003). PCR conditions consisted of 25-µL total volume including 10 µL of MasterMix (5 PRIME, Gaithersburg, MD), 1 µL of each primer, 2–4 µL of total genomic DNA, and water. The thermal cycling profile began with an initial denaturation at 94 °C (10 min) followed by 10 cycles of 94 °C (1 min), 48 °C (1 min), and 65 °C (2 min) (decreased by 0.5 °C per cycle). This protocol was followed by 35 cycles of 94 °C (1 min), 52 °C (1 min), and 65 °C (2 min) and then a final extension of 65 °C (10 min). Amplified fragments were purified using ExoSAP-IT (USB Corporation). Sequencing of those samples was performed at the University of Florida ICBR DNA Sequencing Core Laboratory using standard fluorescent cycle-sequencing PCR reactions (ABI Prism Big Dye terminator chemistry; Applied

Biosystems). The forward and reverse sequences were edited and then aligned using the MUSCLE alignment algorithm in Geneious 6.1.6 (http://www.geneious.com; Kearse et al. 2012) and confirmed by eye. Consensus sequences were generated for each sample using both forward and reverse sequences.

The same individuals were not represented in both mitochondrial datasets, so the 2 loci were analyzed separately. We ran phylogenetic Bayesian inference analyses in MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003) and performed maximum likelihood analyses using RAxML 8.2.4 (Stamatakis 2014). Both MrBayes and RAxML analyses were conducted using the CIPRES Gateway (Miller et al. 2010). We used MrModelTest 2.4 (Nylander 2004) to select models of evolution for both cyt *b* and the control region. We utilized the Akaike information criterion to select the most appropriate model (Posada and Buckley 2004). The GTR+I+G model was selected to use with both genes analyzed.

For the Bayesian analyses, 2 independent runs were initiated with random starting trees and an initial melting point of 0.25, and were run for at least 9 x 10^6 generations with 4 incrementally heated chains (Metropolis-coupled MCMC; Huelsenbeck and Ronquist 2001) sampled every 100 generations. We assessed convergence and stationarity using Tracer version 1.6 (http://beast.bio.ed.ac.uk/Tracer, accessed 23 July 2015); we discarded any trees generated before achieving stationarity. For the maximum likelihood analyses in RAxML, we employed the GTRCAT model for the bootstrapping phase and the GTRGAMMA model for the tree inference phase, which are the default parameters. We calculated sequence divergence values for both cyt *b* and the control region in MEGA6 (Tamura et al. 2013) using the Kimura 2-Parameter model (Kimura 1980), which calculates the average pairwise genetic distances between individuals or groups.

Results

Microsatellite diversity

White-tailed Deer populations from the southeastern United States, represented by 5 or more individuals, were highly polymorphic, with an average of 5.46 alleles per locus and an average H_O and H_E of 0.64 and 0.72, respectively (Table 1). Deer from the Florida Keys showed less genetic diversity than the other deer populations, with an average of 2.88 alleles per locus, 0.36 H_O and 0.5 H_E (Table 1). Moreover, Key deer had the highest value of F_{IS} (0.29). Deer from Curaçao had a F_{IS} value not significantly different from zero, while the estimate of F_{IS} for NSGB deer was the third lowest value recorded (0.04). Southeastern US deer populations had high F_{IS} values and a high number of alleles (Table 1). NSGB and Curaçao showed low values of allelic richness (*AA* = 1.30 and 1.54, respectively) compared with Key Deer (*AA* = 2.29). Linkage disequilibrium was estimated for all marker pairs across all populations, and no significant differences were found. Populations were largely in Hardy–Weinberg equilibrium for all loci, with a few exceptions (4 loci for Big Pine Key population, 2 loci for Joe Budd WMA, 2 loci for St. Vincent WMA, and 4 loci for Scotch WMA).

For the population pairwise values of Θ_{WC}, all the comparisons involving the Key Deer population were significant, indicating the unique genetic signature of these deer (see Table S2 in Supplemental File 1, available online at http://www.eaglehill.us/CANAonline/suppl-files/c173-Mathis-s1). The maximum value of Θ_{WC} between Key Deer and any of the US White-tailed Deer populations was 0.308 and the Θ_{WC} between Key Deer and NSGB deer was 0.691. No comparison was conducted involving the Curaçao deer due to low sample size. Most of the comparisons between NSGB and any of the continental US populations were significant with a minimum Θ_{WC} of 0.545.

All individuals were assigned with high probability to 3 distinct genetic clusters ($\Delta K = 118.27$, $K = 3$; Fig. 2A,; see also Fig. S1 in Supplemental File 1, available online at http://www.eaglehill.us/CANAonline/suppl-files/c173-Mathis-s1): one consisting of deer from the southeastern United States, one representing the deer population of the Florida Keys, and a third distinct cluster composed of deer from NSGB and Curaçao. In the PCoA analysis, the first principal coordinate explained

Table 1. Microsatellite diversity analyses for 17 populations of *Odocoileus virginianus* (White-tailed Deer) sampled at 8 microsatellite loci. Sampled populations include the southeastern United States (Florida, Georgia, Alabama, and South Carolina), *O. v. clavium* (Key Deer) from the Florida Keys, Naval Station Guantanamo Bay (NSGB), and Curaçao. n = sample size; n_A = number of alleles; AA = allelic richness; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = index of inbreeding of individuals resulting from the non-random union of gametes within a subpopulation. * indicates F_{IS} significant at Bonferroni corrected alpha level of 0.00037, corresponding to a nominal alpha level of 0.05. N/A = data not available.

Geographic region/site	n	n_A	AA	H_O	H_E	F_{IS}
Florida Keys						
Big Pine Key, FL	20	2.88	2.29	0.36	0.50	0.29*
Southeastern USA						
Bull Creek WMA, FL	1	N/A	N/A	N/A	N/A	N/A
Citrus WMA, FL	10	4.75	3.75	0.52	0.64	0.20
Corbett WMA, FL	7	5.50	4.44	0.65	0.71	0.10
Eglin AFB, FL	7	5.75	4.89	0.62	0.76	0.20
Joe Budd WMA, FL	14	6.38	4.56	0.67	0.77	0.13
Ocala WMA, FL	7	5.17	4.47	0.69	0.69	0.01
St. Vincent Island NWR, FL	8	4.63	3.73	0.58	0.68	0.15
Three Lakes WMA, FL	6	4.75	4.51	0.71	0.80	0.11
Scotch WMA, AL	12	7.00	4.44	0.61	0.74	0.18*
Washington Co., AL	5	4.63	4.28	0.71	0.76	0.06
Fort Benning, GA	8	4.88	4.02	0.66	0.71	0.09
Harris Neck NWR, GA	10	5.75	3.98	0.58	0.68	0.15
Carlisle WMA, SC	10	5.88	4.14	0.66	0.70	0.07
Chester, SC	10	6.00	4.28	0.70	0.73	0.05
Greater Antilles						
NSGB	20	1.38	1.30	0.35	0.37	0.04
South America						
Curaçao	5	1.57	1.54	0.60	0.45	-0.39
Total/average	160	15.63	4.98			

39.11% of the variability and separated individuals into 2 groups: one formed by mainland United States and Key Deer populations, and the second group composed of deer from NSGB and Curaçao (Fig. 2B). The second principal coordinate explained 22.17% of the variability and differentiated the population of Key Deer from the mainland United States population.

The unrooted neighbor-joining tree based on D_{ps} distances revealed the NSGB deer population clustered with White-tailed Deer from Curaçao with 100% bootstrap support (see Fig. S2 in Supplemental File 1, available online at http://www.eaglehill.us/CANAonline/suppl-files/c173-Mathis-s1). According to this tree, the Key Deer population and continental US deer formed a cluster with 71% bootstrap support. Within this cluster, populations are not well supported with the exception of 2 populations from Florida and 2 populations from South Carolina (Fig. S2).

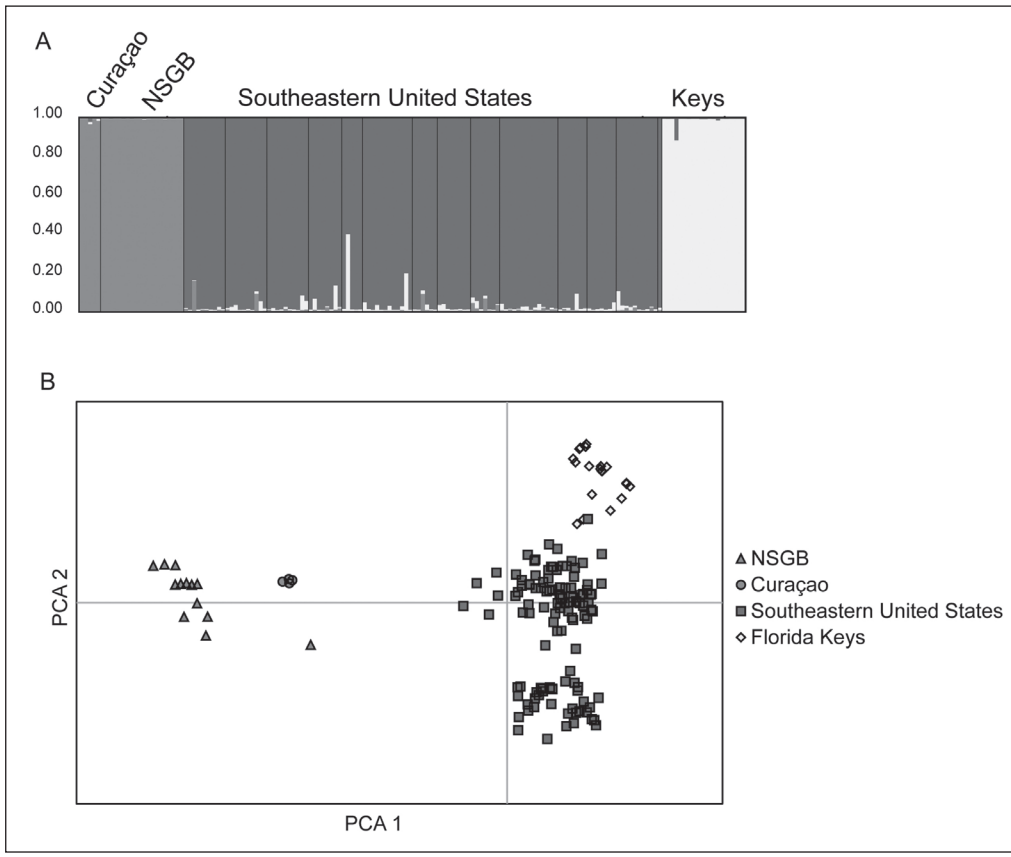

Figure 2. Population structure and principal coordinates analysis (PCoA) of *Odocoileus virginianus* (White-tailed Deer) populations based on nuclear genetic diversity. Populations sampled are from the Naval Station Guantanamo Bay (NSGB), southeastern United States (including the Florida Keys), and Curaçao. (A) Assignment of individuals to $K = 3$ nuclear genetic clusters inferred from STRUCTURE simulations. (B) Distributions of points in the 2 primary dimensions resulting from PCoA analyses conducted using pairwise microsatellite distance values for sampled White-tailed Deer populations.

Mitochondrial analyses

For the cyt *b* gene, Kimura 2-Parameter (K2P) average pairwise genetic distances calculated from the sequences for the NSGB White-tailed Deer ranged from 0.9% (NSGB/French Guiana) to 4.1% (NSGB/Key Deer) (Table 2). The average K2P distance between Mexican individuals and the NSGB deer was 2.7% (Yucatán) and 3% (Tamaulipas). For the control region, average K2P distances were higher than those for cyt *b*, ranging from 2.9% between the NSGB deer and Venezuela individuals to 11.5% between the NSGB deer and those from Tamaulipas (Table 2). The average distance between NSGB and French Guiana, the only South American location represented in both datasets, was 5%. Deer from NSGB and those from the Florida Keys were 7.7% divergent, while NSGB deer and Yucatán deer were 10.1% divergent.

For the 20 NSGB deer examined, there was only 1 haplotype each for both the cyt *b* gene and the control region. Therefore, only 1 individual was used for the final phylogenetic analyses. Maximum likelihood and Bayesian analyses for both genes resulted in the same topologies; only the Bayesian tree is presented for each gene. For the cyt *b* analysis (1019 bp), NSGB deer formed a highly supported sister relationship with White-tailed Deer from French Guiana (posterior probability [pp] = 1, bootstraps [bs] = 99; Fig. 3). The Key Deer formed a strongly supported sister

Table 2. Average (standard error in parentheses) pairwise Kimura 2-parameter genetic distances calculated for cytochrome b (above diagonal) and the control region (below diagonal) for *Odocoileus virginianus* (White-tailed Deer) sampled from Naval Station Guantanamo Bay (NSGB) and other localities in North, Central, and South America. An "x" indicates no samples were sequenced at that gene.

	Mainland USA	Florida Keys	NSGB	Tamaulipas	Yucatán	Guatemala	French Guiana	Curaçao	Venezuela.
Mainland USA	-	0.037 (0.006)	0.030 (0.005)	0.015 (0.002)	0.031 (0.005)	x	0.027 (0.005)	x	x
Florida Keys	0.082 (0.011)	-	0.041 (0.007)	0.041 (0.006)	0.040 (0.007)	x	0.042 (0.007)	x	x
NSGB	0.100 (0.014)	0.077 (0.015)	-	0.030 (0.005)	0.027 (0.006)	x	0.009 (0.003)	x	x
Tamaulipas	0.083 (0.010)	0.105 (0.016)	0.115 (0.018)	-	0.030 (0.005)	x	0.027 (0.005)	x	x
Yucatán	0.095 (0.013)	0.078 (0.014)	0.101 (0.018)	0.094 (0.015)	-	x	0.028 (0.006)	x	x
Guatemala	0.091 (0.013)	0.074 (0.014)	0.084 (0.017)	0.106 (0.017)	0.056 (0.011)	-	x	x	x
French Guiana	0.114 (0.015)	0.091 (0.016)	0.050 (0.011)	0.126 (0.020)	0.116 (0.020)	0.099 (0.018)	-	x	x
Curaçao	0.089 (0.012)	0.066 (0.013)	0.037 (0.009)	0.104 (0.017)	0.087 (0.016)	0.079 (0.015)	0.056 (0.012)	-	x
Venezuela	0.098 (0.012)	0.079 (0.013)	0.029 (0.005)	0.113 (0.016)	0.096 (0.015)	0.084 (0.014)	0.057 (0.010)	0.033 (0.006)	-

relationship with respect to all the other White-tailed Deer in the analysis (pp = 0.95, bs = 80).

Maximum likelihood and Bayesian analyses of the control-region data (521 bp) placed the NSGB sequence within a well-supported South American clade of deer from Venezuela, Curaçao, and French Guiana (pp = 0.96, bs = 78; Fig. 4). The NSGB deer was sister to a Venezuelan individual (pp = 0.92, bs = 91). A strongly supported clade of deer from the southeastern United States and the Florida Keys also was recovered (pp = 1, bs = 96), separate from another clade of deer from Canada, mainland United States, and Tamaulipas, Mexico (Fig. 4). Yucatán individuals along with an individual from Guatemala formed a polytomy with respect to the Venezuelan clade and the southeastern United States clades.

Discussion

The nuclear and mitochondrial analyses presented here indicate that deer sampled from within the boundaries of NSGB did not originate from the United States as has been suggested, or from Mexico as de Vos et al. (1956) reported. Rather, these genetic analyses show a closer genetic relationship with White-tailed Deer lineages from northern South America and Curaçao, which geologically lies on the

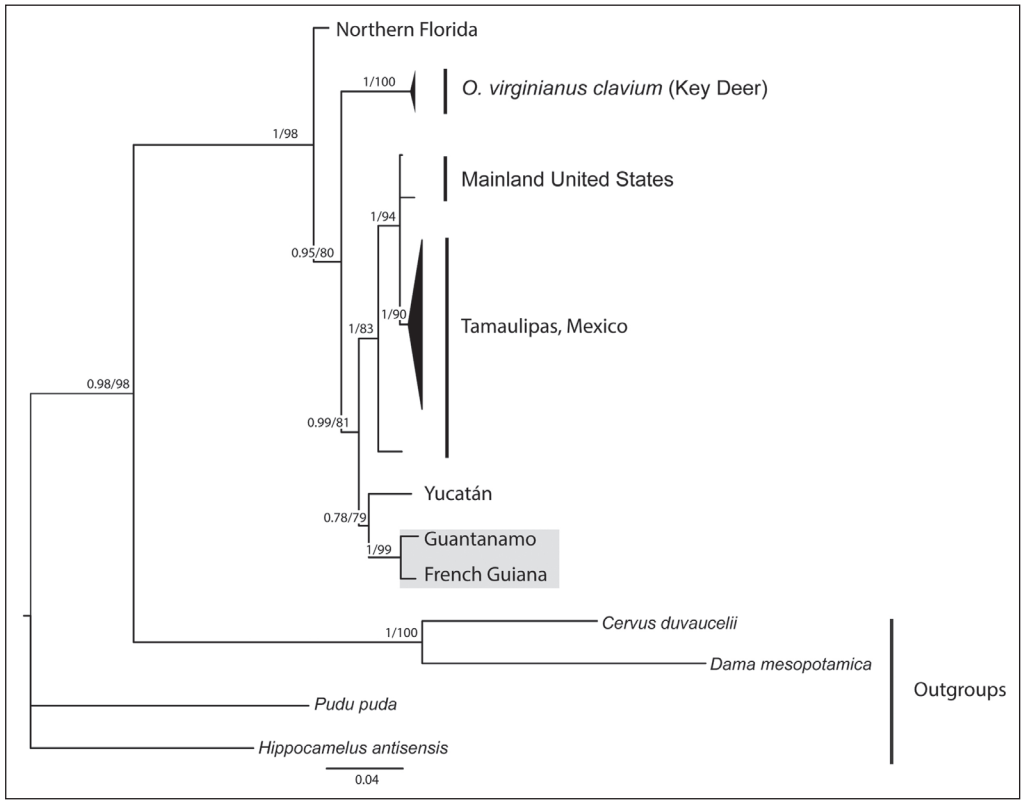

Figure 3. Bayesian phylogenetic tree of the cytochrome *b* gene (cyt *b*) for *Odocoileus virginianus* (White-tailed Deer). Tips are collapsed for major clades. Support values are given at the nodes as posterior probability/maximum likelihood bootstrap value.

continental shelf of South America. This casts doubt on the story of deer arriving at NSGB with American servicemen in the mid-20th century; at the very least if they did bring deer onto the base, those deer did not contribute to the gene pool of the current population.

None of the microsatellite analyses (PCoA, STRUCTURE, and distance-based tree) supported the long-held assumption that NSGB White-tailed Deer are derived from the mainland United States or the Key Deer populations. Instead, results strongly support a closer relationship with Curaçao White-tailed Deer. Moreover, the microsatellite data presented provide the first insight into the genetic diversity among the deer populations analyzed herein.

As expected, our microsatellite analysis indicates that Key Deer have low levels of genetic variation and high F_{IS}, suggesting a certain level of inbreeding. This finding is consistent with previous studies based on mitochondrial diversity that showed a lack of nucleotide variation at the mitochondrial level and a high differentiation with peninsular/mainland Florida populations (Ellsworth et al. 1994). This pattern is expected under a model of island population after a founder event (Mayr 1954). However, we did not find the same genetic pattern in the deer populations from other islands, since NSGB presented a very low F_{IS} and the Curaçao F_{IS} was

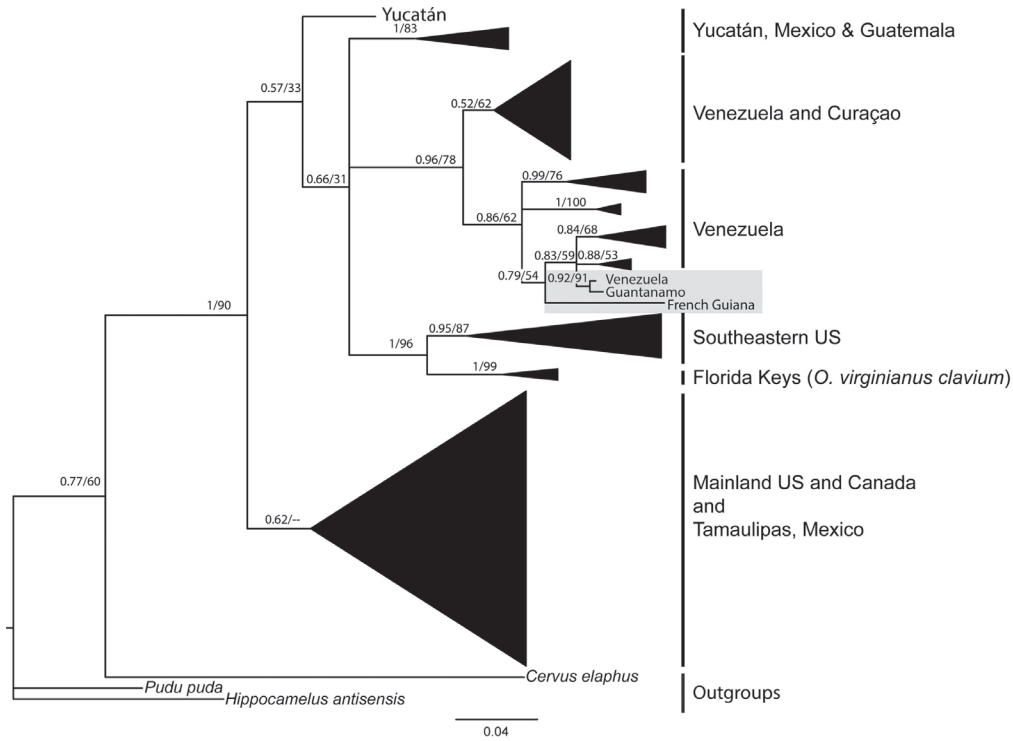

Figure 4. Bayesian phylogenetic tree of the control region for *Odocoileus virginianus* (White-tailed Deer). Tips are collapsed for major clades. Support values are given at the nodes as posterior probability/maximum likelihood bootstrap value. The grey box highlights the location of the Guantanamo Bay White-tailed Deer.

not significantly different from zero; both of these signatures are ones that would be expected for populations with no inbreeding. In the case of deer from NSGB, the current levels of genetic variation and lack of apparent inbreeding suggest that this population did not arise as a result of a single founder event involving a small number of individuals. Curaçao deer were reportedly brought to the island by Native Americans in pre-Columbian times, likely on many occasions for over 500 years (van Buurt 2010). Considering the close proximity between Curaçao and Venezuela (60 km), and the shared fauna and flora, our data are concordant with multiple introductions of deer from northern South America, and/or natural colonization from the same area via the Lesser Antilles. However, NSGB and Curaçao did show low values of allelic richness, whereas the mainland southeastern deer had high numbers of alleles. One potential cause for these differences in allelic richness could be sample size. While F_{IS} is more influenced by the frequency of alleles, allelic richness is influenced more by the number of alleles, allowing rare alleles to carry weight and resulting in estimates that may be biased, based on their sample sizes (Greenbaum et al. 2014, Leberg 2002). The results from the F_{ST} analogue of Θ_{WC} are consistent with the other genetic analyses, indicating a great differentiation of the NSGB deer from all the US deer (Key Deer and continental deer). Although the Wahlund effect was not detected through the STRUCTURE analysis for the mainland populations, the allelic diversity detected was consistent with previous studies of southeastern US deer (DeYoung et al. 2003). Many mainland United States populations may also be admixed due to historical introductions, which could account for the lack of subpopulation structure. Due to sample-size limitation, these observations should be taken with caution and re-evaluated in future studies when more samples and more localities along the Caribbean islands can be included.

The mitochondrial data, which increases the geographical sampling from the United States, NSGB, and Curaçao in the microsatellite dataset to include Mexico, Central America, and South America, are congruent with the microsatellite data indicating deer found at NSGB are not closely allied with North America. In both the cyt *b* and control-region datasets, a South American individual is sister to the NSGB deer, despite the fact that the same localities were not sampled in both datasets. French Guiana was the only South American locality represented in both datasets and the only South American locality for the cyt *b* dataset. Of the 3 South American localities in the control-region dataset (French Guiana, Venezuela, and Curaçao), Venezuela showed the closest genetic affiliation with NSGB. Venezuelan White-tailed Deer are genetically and morphologically distinct from North American deer, with pairwise genetic distances similar to species-level differences among other cervids (Molina and Molinari 1999, Moscarella et al. 2003). Our results are consistent with these previous studies in that the control-region data support a distinct South American clade, with NSGB nestled among samples from Venezuela, Curaçao, and French Guiana. These data suggest that northern South American and NSGB populations have more recent common ancestry than the other populations analyzed. However, the control region does not provide strong overall phylogenetic resolution, resulting in a polytomy with respect to the placement of clades from

the United States, the Yucatán, Guatemala, and South America. Furthermore, deer from the United States are paraphyletic with respect to other deer from the United States as well as eastern Mexico and Canada. Average pairwise mitochondrial genetic distances between NSGB and the South American sequences were lowest among all other comparisons, whereas pairwise differences were highest between NSGB deer and those from Mexico.

Speculation that NSGB deer came from the Florida Keys was likely due to the geographical proximity and the similar diminutive size of NSGB deer. Although the genetic analyses rule out the Keys as a source of NSGB deer population, reduction in body size could be the product of similar selection pressures. Evolutionary change may occur rapidly on islands, especially morphological change (Evans et al. 2012, Millien 2006). White-tailed Deer also have demonstrated fairly rapid phenotypic evolution in response to diet (Monteith et al. 2009, Simard et al. 2008). The smaller size of the NSGB deer compared to mainland deer may be a result of the island effect, as seen in other deer found in the Caribbean (Heffelfinger 2011, Seaman 1966), Curaçao, and in the Key Deer. Determining precisely when and from where deer arrived in Cuba requires more sampling and would permit an estimate of how much time it might take for the establishment of island dwarfism in a large mammal.

How South American White-tailed Deer came to NSGB is not precisely known, although direct transport of deer from Venezuela to Cuba is a strong possibility. Along with Cuba, White-tailed Deer populations are established on Jamaica, the US Virgin Islands, and Puerto Rico, along with unverified reports of deer on other islands in the West Indies (Heffelfinger 2011). Deer populations in Puerto Rico and Jamaica appear to be recent introductions during the 1960s and 1980s, respectively, while those in the Virgin Islands are attributed to the Dutch in the 1700s (Heffelfinger 2011, Seaman 1966). In addition to the ones mentioned previously, Borroto-Páez and Woods (2012) list the Dominican Republic, Antigua, Barbuda, Romano, Sabinal, Dominica, Grenada, Guajaba, and Hispaniola as having deer, although there is little to nothing known about the status, size, or health of those populations. Whether deer colonized those islands from South America via island-hopping, directly via human-aided translocation, or some combination of both, remains untested. On Curaçao, archaeological excavations suggest deer were likely brought over by Caquetío Indians, a tribe that arrived from Venezuela sometime around 500 AD. They were reported as being common on the island in the mid-1800s and are still found today in smaller numbers (van Buurt 2010). They are classified as their own protected subspecies (*O. v. curassavicus* (Hummelinck)). Native Americans used White-tailed Deer extensively for clothes, food, and other items both practical and ceremonial (e.g., bone flutes made from deer bones found on the Los Roques archipelago; Newsom and Wing 2004): thus, human-aided movement of the deer throughout the Antilles is highly probable. The timing and precise route requires additional sampling from islands of the West Indies.

Unassisted movement of these deer through the Lesser Antilles and Greater Antilles could still be a possibility. Deer are known to be strong swimmers, moving easily between islands (Heffelfinger 2011). In the West Indies, ocean currents moving in a

mostly east-to-west direction means most flotsam arrives via South America; the evolutionary history of many reptiles and amphibians in the West Indies agrees with this possible mode of colonization (Hedges 1996, 2006). However, the lack of historical records of deer from the time of European contact coupled with absence of other naturally established populations of large mammals on nearby islands casts doubt on natural colonization from South America via island-hopping by deer. Further, the relatively low mitochondrial genetic distances between South America and NSGB deer coupled with the strong genetic clustering seen in fast-evolving microsatellite data are not fully supportive of a natural colonization scenario.

Because there is no published evidence of established populations of White-tailed Deer in the Lesser Antilles aside from unverified reports (Borroto-Páez and Woods 2012, Heffelfinger 2011), population surveys and sampling for *Odocoileus* throughout the Caribbean basin are necessary to fully complete the picture of where, when, and how deer arrived in Cuba and NSGB. It also remains unknown whether the deer sampled in NSGB are derived from deer outside the base. The base was founded in 1903, and since that time deer inside the base have increased in population number to the point of being a nuisance, whereas outside the base they are very rare (G. Silva, National Museum of Natural History, Havana Cuba, and R. Borroto-Páez, Cuban Zoological Society, Havana, Cuba, pers. comm.).

Future research could estimate migration rates and gene flow among known deer populations on Caribbean islands as well as determine the precise colonization route of White-tailed Deer. Lastly, future research should determine whether the deer within NSGB and in Cuba are from the same stock and whether they represent populations in need of protection and management. With the possibility of continued easing of diplomatic relations in Cuba and the opening of the country, especially for science, it is very important to evaluate the current biodiversity and the origin of species on the island before any management program is established.

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