Protocol Development for Genetic Differentiation of Wild and Pen-Raised White-tailed Deer

Graduate Research Assistant:

Jordan Youngmann

Principal Investigators:

Steve Demarais and Bronson Strickland, Forestry and Wildlife Research Center, Mississippi State University, and

Randy DeYoung, Caesar Kleberg Wildlife Research Institute, Texas A&M U – Kingsville

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CHAPTER 1

LANDSCAPE GENETICS AND THE INFLUENCE OF NORTHERN STOCK SOURCES ON FREE-RANGING WHITE-TAILED DEER IN SOUTHCENTRAL UNITED STATES

INTRODUCTION

Translocation of wildlife to restore species to their original range is a common practice in conservation ecology. Unfortunately, consideration hasn't always been given to genetic consequences of non-native introductions to wild populations (Leberg 1990). Introduction of novel genetic variation through translocations can result in loss of diversity through founder effects and the replacement or dilution of locally adapted gene complexes and, in rare cases, outbreeding depression (Avise 1994, Allendorf et al. 2001). Consequently, translocation events often result in a measurable change in the genetic diversity and structure of restored populations for generations (DeYoung et al. 2003a, Seidel et al. 2013).

White-tailed deer populations across the United States had reached all-time lows by the early 1900s due to unregulated harvest and habitat loss. To restore deer populations, state agencies captured and translocated deer from as far away as Michigan, Wisconsin, and Mexico during the 1930s through 1960s (Leopold 1929, Cook 1943, Blackard 1971). Previous genetic studies of white-tailed deer populations in the southeastern US have found admixture, where some patterns of genetic structure were congruent with historic stocking efforts, whereas remnant populations of native deer recovered in other areas (Leberg et al. 1994, Leberg and Ellsworth 1999, DeYoung et al. 2003a). Although indications of genetic bottlenecks or founder effects were apparent, high levels of allelic diversity and heterozygosity reflect admixture and rapid recovery to pre-decline population levels (DeYoung et al. 2003a). However, there are

indications that certain source stocks may have affected physical traits, such as breeding phenology (Sumners et al. 2015).

Throughout the restoration period, state agencies acquired stock sources from out-of-state when they could not procure local deer for translocation. Louisiana, Mississippi, and Alabama brought deer from Wisconsin and Michigan to augment their restocking efforts (Blackard 1971). This use of northern stock sources resulted in the colloquial assumption that certain geographic regions contain remnant northern deer stocks (Johnathan Bordelon, LDWF, Chris Cook, ADCNR, and William McKinley, MDWFP, *personal communication*). However, intolerance of northern stocks to southern diseases and parasites, such as epizootic hemorrhagic disease (EHD), has suggested that survival rates of northern deer would have been limited after translocation (Lukefahr and Jacobson 1998, Gaydos et al. 2002). Furthermore, large-bodied northern deer with heavy coats may not have fared well in southern climates. The many repeated restocking efforts suggest poor success of early releases after initial translocations (Blackard 1971). Circumstantial evidence indicates that northern stocks did not contribute to the variable breeding dates found in the southeastern U.S. (Sumners et al. 2015). However, previous studies have not directly evaluated potential genetic legacies of northern stock sources.

Little is known about phylogenetic structure of white-tailed deer populations pre-dating human influence through restocking. Currently, the 38 recognized subspecies of white-tailed deer across the Americas are based on morphometric, not genetic, differences (Heffelfinger 2011). Ellsworth and colleagues (1994) addressed pre-restoration genetic structure in deer and found that mtDNA haplotype groups did not match subspecies delineations, but instead were congruent with geographic regions affected by the last glacial maxima during the Pleistocene. This genetic structure was apparent despite the presence of admixture from recent human

intervention through restocking (Leberg and Ellsworth 1999). Further research that expands the geographic scope of phylogenetic inquiry may help to better understand population structure of white-tailed deer across their range.

My overall goal was to better understand the genetic impacts caused by historical releases of white-tailed deer. I assessed the genetic diversity and structure of free-ranging white-tailed deer from Louisiana, Mississippi, and Alabama and stock source populations from Mexico, Texas, Wisconsin, Michigan, and North Carolina. I hypothesized that free-ranging populations are admixed and structured according to historic stocking efforts (Leberg et al. 1994, Leberg and Ellsworth 1999, DeYoung et al. 2003a, Sumners et al. 2015). I also included outgroup populations from Oklahoma, New York, and Florida to better understand the genetic structure of white-tailed deer across a broad portion of their range. Finally, to better evaluate the influences of northern stock sources, I compared populations that had received northern stock sources to deer sampled from Wisconsin and Michigan. I hypothesized that I wouldn't find evidence of northern genetic variation in areas where they were stocked due to low fitness in southern climates and low resistance to regional disease (Lukefahr and Jacobson 1998, Gaydos et al. 2002).

STUDY AREAS

Southcentral Populations

The sample area consisted of Louisiana, Mississippi, and Alabama. All 3 southcentral states used native, remnant stocks when possible but also used more geographically distant populations as well—sometimes as far away as Michigan, Wisconsin, and Mexico (Blackard 1971: Table 1). Additionally, as was the case with Leaf River Refuge, MS, initial translocations allowed for sufficient recovery to be used as a stock source. I, therefore, conducted within-state sampling to

best capture both native lineages and the potential influences of non-native stock used during restoration. Blackard (1971) lists native and non-native stock source populations and release sites within each state. However, records are listed by county, with large variation in known source stock, number of deer translocated, and exact release locations. Due to the lack of these specifics, I only sampled counties or parishes where known source populations and numbers of deer stocked. When possible, sampling was conducted within a 16-km buffer around known stocking locations. In instances of unknown stocking locations, I limited my sampling to a 16-km buffer around the geographic center of the county. Additionally, I included samples from DeYoung et al. (2003a) and Sumners et al. (2015) where appropriate.

Outgroup Populations

Outgroups serve as references for potentially admixed populations and included significant sources used for historical relocations. At least 110 deer were translocated from Iron Mountain, Michigan to Alabama. At least 150 deer were shipped to Louisiana and 158 to Mississippi from the Sandhill Wildlife Area, Wisconsin and at least 25 deer were brought to Alabama and 65 to Louisiana from Texas. The historic Pisgah Game Preserve in North Carolina provided 76 deer to Alabama and 35 to Mississippi. I obtained samples from the Iron Mountain area of Michigan (hereafter, Michigan), the Sandhill Wildlife Area in Wisconsin (hereafter, Wisconsin), 3 Texas populations, and the Biltmore Estate (part of the historic Pisgah Game Preserve) and surrounding areas in North Carolina. Additionally, I obtained samples from the Adirondacks in New York and from Joe Budd WMA, Florida (hereafter, Florida) due to recorded stocking of the Pisgah Game Preserve, NC (hereafter, North Carolina) with Adirondack (hereafter, New York) and Florida deer in the early 1900s. Finally, I included samples from the Noble Foundation, OK

(hereafter, Oklahoma) to provide further geographic coverage. Samples from Mexico, Texas, Oklahoma, and Florida and included those previously analyzed by DeYoung et al. (2003a).

METHODS

DNA Extraction and Amplification

Each sample was placed into a labeled bag and frozen. Samples were then transferred on ice to Mississippi State University and stored at -20ºC until DNA extraction. Extracted samples were stored at -80ºC. I isolated DNA from tissue samples using the Qiagen® DNeasy™ Tissue Kit (QIAGEN Genomics Inc., Hilden, Germany), following the manufacturer's protocol. I amplified 15 microsatellite DNA loci , including BL25, BM4208, BM6438, BM6506, BM848, Cervid1, ILSTS011, INRA011, and OarFCB193, D, K, N, O, P, and Q (Anderson et al. 2002, DeYoung et al. 2003b). I amplified loci in 4 multiplex reactions, as described by Anderson et al. (2002) and DeYoung et al. (2003a), and loaded the resulting products onto an automated genetic analyzer for separation and detection (3130*xl*, Applied Biosystems, Foster City, CA). I determined allele size calls for each locus using GeneMapper 4.0 (Applied Biosystems).

Data used from DeYoung et al. (2003a) and Sumners et al. (2015) had been collected on a different sequencing platform than the one I used. To ensure that differences in migration of fragments did not affect allele size calls, I amplified and genotyped 71 individuals from those 2 studies chosen to be representative of the distribution of alleles detected in the studies. I used those genotypes to calibrate allele bins to ensure that microsatellite size-calls matched between datasets. I could not consistently assign allele calls for the Q locus, so I omitted that locus from further analyses.

Data Analysis

I calculated summary statistics for all populations, including gene diversity (*H*; Nei 1987) and the inbreeding coefficient (F_{IS}, Weir and Cockerham 1984) using FSTAT (Goudet 1995, 2002). I tested for Hardy-Weinberg equilibrium and linkage disequilibrium within populations and by locus using ARLEQUIN 3.1 (Excoffier et al. 2005). I corrected for multiple comparisons using a Bonferroni procedure. Population genetic theory articulates that recent admixture between different populations can inflate F_{IS} and linkage disequilibrium and, therefore, reveal historic introgression of stock sources in deer (Wahlund 1928).

To assess population genetic structure, I tested differentiation among populations using Nei's genetic distance (D_A; 1983) with the R-based software package, ADEGENET (Jombart et al. 2010). Nei's D_A assumes no underlying evolutionary model, unlike F_{ST}, which may not perform well in the presence of admixture (Nei and Kumar 2000). It is, therefore, better suited for analysis of populations wherein lineage mixing has occurred.

All populations

The theory of isolation by distance postulates that there will be a positive relationship between the geographic distance and genetic differentiation of populations as a result of finite dispersal limiting gene flow between individuals (Wright 1943). Therefore, populations separated by larger geographic distances should have greater genetic differentiation than populations closer together. However in populations that have received translocated stock sources, this structure should not be observed.

To test for isolation by distance, I conducted a Mantel test (Mantel 1967) using APE software package (Paradis et al. 2004) implemented in Program R (R Core Team 2015), including all 73 populations. A Mantel test estimates the correlation between matrices of pairwise geographic and genetic distances (kilometers and Nei's D_A, respectively) and should be

positive under isolation by distance. I also calculated slope and R^2-value between geographic and genetic distances to determine the strength of this correlation. I assessed statistical support based on 10,000 permutations of rows and columns.

I conducted population assignments across all populations using a Bayesian clustering algorithm implemented in the computer program STRUCTURE, version 2.3 (Pritchard et al 2000). For initial runs, I used the admixture model with correlated allele frequencies, assuming that some individuals may have ancestry from > 1 subpopulation. Additionally, I used the LOCPRIOR option to designate the 10 outgroup populations as knowns. The LOCPRIOR model allowed the use of the sampling location of a population as a Bayesian prior to better inform clustering of groups. I modeled the additional 63 populations as unknowns. This method allowed me to use location information to inform the structuring of outgroups and further clarify which of those populations may have contributed to the genetic structure of free-range, southcentral populations. Finally, I configured the ancestry prior at 1/K (1/73 = 0.014) to more accurately assess ancestry proportions of samples from unbalanced population sizes, as suggested by Wang (2016).

I conducted runs consisting of 50,000 Markov Chain Monte Carlo (MCMC) repetitions as a burn-in to minimize the effects of the starting configuration, followed by 150,000 repetitions for K = 1 through 10 hypothesized genetic clusters; I performed 5 replicate runs at each K to assess consistency among runs. I determined the most likely number of genetic clusters represented within my data using Evanno et al.'s (2005) ΔK method, implemented in the software program STRUCTURE HARVESTER (Earl and vonHoldt 2012). After inferring a most likely K-value, a longer run of 5 iterations of a 100,000 MCMC burn-in followed by 1,000,000 repetitions at K, K+1, and K-1 was performed. I used the results from this second run

for further analyses. I visualized the STRUCTURE output using the web interface CLUMPAK which combines the results of replicate runs (Kopelman et al. 2015).

Comparison of Northern Stock Sources with Recipient Southcentral Populations

To determine if the use of northern stock sources has had a lasting impact on the genetic variation found in the southcentral populations, I performed an analysis of molecular variance (AMOVA) between a group consisting of the 2 Midwest stock sources, Wisconsin and Michigan. I performed the AMOVA using ARLEQUIN 3.1 (Excoffier et al. 2005). To test for the presence of isolation by distance, I conducted a Mantel test (Mantel 1967) with 10,000 permutations between rows and columns through the APE software package (Paradis et al. 2004) implemented in Program R (R Core Team 2015), across southern U.S. populations that had received historic restocking from Midwest stock sources and their Midwestern stock sources. If the Midwest stock sources had no lasting impact on the southern genetic variation, I'd expect to see isolation by distance present.

To assess the presence of Midwest genetic variation, I also conducted population assignments for southcentral populations located near known stocking locations of northern deer. For initial runs, I used the admixture model with correlated allele frequencies, assuming that some individuals may have come from > 1 subpopulation. Additionally, I used the LOCPRIOR model wherein I designated both Midwest stock sources, Wisconsin and Michigan, as known locations. I then modeled the 14 southcentral populations identified as having potential remnants of northern deer as unknown locations (Table 1). Finally, I configured the ancestry prior at 1/K (1/16 = 0.0625) to more accurately assess ancestry proportions of samples from unbalanced populations sizes (Wang 2016). I then conducted an initial run to determine the most likely K followed by a longer K, K+1 and K-1 run to produce the results.

Comparison of West and East Regions

I found evidence of a west-east split along the Mississippi River when I conducted preliminary analysis of all 73 populations. There is evidence in the literature of similar longitudinal genetic structure along river systems in other southeastern U.S. species (Bermingham and Avise 1986, Avise 1992, Gill et al. 1993, Gill et al. 1999). Therefore, I performed an analysis of molecular variance (AMOVA) to quantify proportions of genetic variation both within and among groups consisting of all populations west of the Mississippi River compared with all populations east of the Mississippi River. I performed the AMOVA using ARLEQUIN 3.1 (Excoffier et al. 2005). To test for isolation by distance, I conducted a Mantel test (Mantel 1967) with 10,000 permutations between rows and columns using the APE software package (Paradis et al. 2004) implemented in Program R (R Core Team 2015) across the west group and the east group, respectively. I also calculated slope and R^2-value between geographic and genetic distances to determine the strength of this correlation.

To further assess genetic structure within regions, I conducted Bayesian clustering analyses using STRUCTURE. As before, I used the admixture, correlated allele frequency, and LOCPRIOR models, where all outgroups were modeled as knowns and southcentral populations, as unknowns. I ran both groups with ancestry priors of 1/K: western group (1/29 = 0.034) and eastern group (1/54 = 0.019) to more accurately assess ancestry proportions of samples from unbalanced population sizes (Wang 2016). I evaluated best fit for K using Evanno's ΔK, and conducted a long run at K-1, K, and K+1.

RESULTS

I analyzed 2,014 free-range individuals from 73 populations across 7 states in the U.S as well as samples from Mexico (Fig. 1). Average gene diversity ranged from 0.60 (Runnells-Pierce Ranch,

TX) to 0.79 (Evangeline Parish, LA), and the inbreeding coefficient (F_{IS}) ranged from -0.05 (Red Dirt Wildlife Preserve, LA) to 0.16 (North Carolina; Table 2). No populations exhibited loci that were out of Hardy-Weinberg equilibrium. However, Runnels-Pierce Ranch, TX had 2 loci that were monomorphic and Catahoula, LA, and Red Dirt, LA, both had 1 locus that was monomorphic. Finally, linkage disequilibrium ranged from 0 to 9, where Juniper Creek, MS had the highest number of pairwise loci in disequilibrium (Table 2).

Overall, all 3 states had similar average F_{IS} and gene diversity levels. Louisiana had the highest average number of loci in linkage disequilibrium ($\bar{x}$ = 2.4, SD = 1.9; Table 2) and Alabama had the lowest ($\bar{x}$ = 1.2, SD = 2.0). When populations were split into a western group and an eastern group, there was a similar lack of difference between summary statistics. The western group had a higher average number of loci under linkage disequilibrium ($\bar{x}$ = 2.7, SD = 2.1) than the eastern group ($\bar{x}$ = 1.9, SD = 2.2).

Pairwise Nei's D_A ranged from 0.068 (Catahoula Preserve, LA, and Simpson County, MS) to 0.420 (Winn Parish, LA, and Lamar County, MS). Genetic differentiation between the two Midwest sources (Wisconsin and Michigan) and southcentral populations averaged 0.204 (SD = 0.044). Average differentiation between southcentral populations west and east of the Mississippi River was 0.224 (SD = 0.053).

All populations

There was no support for isolation by distance over all 73 populations (P = 0.722, R^2 = 0.001, slope = 2.9×10^{-6}). Pairwise relationships with Winn Parish, LA, and Upper Sardis, MS, tended to show high levels of genetic differentiation regardless of geographic distance (Fig. 2).

The ΔK for the preliminary STRUCTURE run of all 73 populations showed a best fit for 2 clusters (Fig. 3). However, there was an additional increase in ΔK at K=5, so I also evaluated

assignments at that level. The analysis revealed distinct clustering between outgroup populations modeled as known populations, but high levels of admixture across all southern populations modeled as unknown populations. Despite these levels of admixture, K=2 results indicated an overall trend of genetic structure on either side of the Mississippi River (Fig. 5). Exceptions to this can be found in Mississippi populations that reside close to the Mississippi River, including Copiah County, Wilkinson, Millbrook, and Amite County. This same west-east split can be seen in the outgroup populations where the southwest and southeast populations cluster separately and the 2 Midwest populations were evenly split in their assignment proportions.

Assignments for K=5 revealed additional structure among outgroups, with Mexico, Wisconsin, and Michigan, beginning to show signs of overfitting (Fig. 5). Similar to my findings at K=2, southern populations west of the Mississippi River grouped with Runnels-Pierce Ranch, TX and Oklahoma along with Copiah County, MS, Millbrook, MS, and Amite County, MS. However, southern populations east of the Mississippi River showed substructure with Noxubee WMA, MS, Walker, MS, East Mississippi Sportsman's Association, MS, and all Alabama populations clustered together.

Comparison of Midwestern Sources with Recipient Southern Populations

An analysis of molecular variance (AMOVA) between groups split into Midwestern populations (Wisconsin and Michigan) and all southern populations found within groups comprised <0.1% of the variation. Variation among populations within groups made up 4.5%, while variation within populations and individuals constituted 95.5%. Isolation by distance over northern stock sources and the recipient southcentral populations was not significant ($P = 0.612$) with a R^2-value of 0.011 and a negative slope of -6.2×10^{-6} (Fig. 3).

Assessment of ΔK for preliminary northern stock source analysis showed a best fit for 3 and 5 clusters (Fig. 4). For K=3, Wisconsin and Michigan clearly grouped together while most of the southern populations split west-east separate from the Midwest populations. However, Union Parish, LA, Tensas NWR, LA and Black Warrior WMA, AL were all admixed with the Midwest group. For K=5, Wisconsin grouped separate from all populations and Michigan showed indications of overfitting. The southern populations split west-east along the Mississippi River. However, Winn Parish, LA and Grant Parish, LA fell out into a distinct group.

Comparison of West and East Subgroups

An analysis of molecular variance (AMOVA) between populations west of the Mississippi River (hereon, southwestern) and populations east of the Mississippi River (hereon, southeastern) showed 94.3% of genetic variation derived from within populations and individuals (Table 3). Variation among populations within groups contributed 4.9%, and variation within groups only comprised 0.9%. Isolation by distance within west and east groups, respectively, revealed the southwestern group had no isolation by distance ($P = 0.771$, $R^2 = 0.014$, slope = -2.7×10^{-5}; Fig. 2). The southeastern group exhibited isolation by distance ($P = 0.001$, $R^2 = 0.090$, slope = 7.7×10^{-5}; Fig. 3).

Assessment of ΔK for all southwestern populations showed a best fit for 2 clusters (Fig. 6). There was also a second, smaller increase in ΔK at K=8, which clustered all southwestern populations distinctly apart from the outgroups, with high levels of admixture. At K=2, all western southcentral populations grouped together, with the Runnels-Pierce, TX and Oklahoma outgroup populations (Fig. 7). Mexico, Double G Ranch, TX, Wisconsin, Michigan, and Florida were all evenly split between both groups. King Ranch, TX, New York, and North Carolina all grouped separately from the southwestern populations.

Assessment of ΔK for all southeastern populations showed a best fit for 2 clusters (Fig. 6). However, there was also a second, smaller increase in ΔK at K=7, which provided finer-scale, biologically feasible population assignments. Therefore, I conducted the long run at K=1 through K=3, as well as K=6 through K=8. Overall assignment at K=2 revealed a west to east split among outgroup populations that fell along the Mississippi River, with Wisconsin, and Michigan evenly split between both groups (Fig. 7). All Mississippi and Alabama populations clustered with the eastern outgroups. However, at K=7, evidence of further sub-structuring became apparent with Noxubee NWR, MS, Walker, MS, Fayette County, AL, Sumter County, AL, and Marengo County, AL all clustering together.

DISCUSSION

Admixture was evident across all southcentral U.S. populations. Even when clear clustering was apparent between populations, STRUCTURE output revealed highly variable ancestry within individuals and throughout most populations. Similarly I found high levels of variation within individuals and populations in AMOVA analyses while genetic variation was low between populations regardless of groupings. Finally, I found low levels of isolation by distance across populations with hierarchical genetic structure explaining low amounts of the variation. Additionally, I found genetic differentiation was highly variable among populations geographically close to each other, indicating admixture. These results mirror multiple findings from genetic analyses of white-tailed deer in the southeast with both DeYoung et al. 2003 and Sumners et al. 2015 reporting admixture. Considering that restoration efforts continued into the 1970s, some populations may be only 10-15 generations removed from translocated stock sources and evidence of genetic mixing is still apparent. To this end, admixture remains the clearest genetic signature of restocking efforts found in southern U.S. deer populations.

Levels of genetic diversity across all populations were similar to those reported by DeYoung et al. 2003. Though they found evidence of bottlenecking in Mississippi populations, high genetic diversity may have been a result of rapid post-restoration population expansion. The higher levels of linkage disequilibrium found in the southwestern populations may be a genetic signature of admixture called the Wahlund effect (Wahlund 1928).

I also found a lack of isolation by distance in populations west of the Mississippi River whereas eastern populations exhibited significant isolation by distance albeit with a low slope and little of the variation explained by geographic separation. Louisiana mostly received stocking from within-state sources along with at least 200 Texas deer and at least 363 Wisconsin deer (Blackard 1971). Mississippi and Alabama actually received more out-state-stock - 6 states in the case of Mississippi and 7 states for Alabama – but a majority (>75%) of translocated individuals came from native, within-state stock (Blackard 1971, McDonald and Miller 2004). It is unclear why the southeastern population displays isolation by distance when the southwestern population doesn't considering that the restocking histories across all 3 states are similar. However, genetic assignments repeatedly revealed grouping of Alabama deer with some Mississippi populations that were geographically proximate to the Sumter, Clarke, and Marengo Counties in Alabama. Of these Mississippi populations, Noxubee NWR and Walker were also included in DeYoung et al.'s (2003) hypothesized naturally regenerated group. All of these populations may be the result of natural regeneration and, therefore, have maintained isolation by distance despite translocation efforts.

Genetic structure in white-tailed deer across southcentral U.S. appears to split west to east along the Mississippi River based on genetic assignment across all populations. Care must be taken when inferring population structure at K =2 as use of Evanno et al.'s (2004) ΔK as an a

priori determination of the mostly likely number of clusters will often settle on 2 groups even in the presence of further genetic substructure (Janes et al. 2017). However, the west-east split that I observed can also be seen in higher K-values over all populations as well as in subsequent sub-analyses. This genetic split may be an artifact of the higher levels of admixture in the west as evidenced by increased linkage disequilibrium and lack of isolation by distance. Another explanation is that this genetic split is a result of using predominately within-state stock sources (Blackard 1971). Genetic structure along the Mississippi River could result from mixing only regional stocks on either side, which could create differentiation between the southwestern and southeastern populations. The presence of isolation by distance in the southeastern populations may indicate that the genetic effects of restocking is beginning to disperse through gene flow.

However, similar east to west interspecific phylogeographic splits have been documented in the southeast U.S. as a result of Pleistocene glaciation, including in the Carolina chickadee and several fish species (Bermingham and Avise 1986, Avise 1992, Gill et al. 1993, Gill et al. 1999). In white-tailed deer, structure due to glaciation has been shown between south and central Florida, the panhandle, and northern Florida into South Carolina (Ellsworth et al. 1994). Genetic structure due to glaciation has also been documented in other large mobile mammals such as the brown bear (*Ursus arctos*) in Europe (Taberlet and Bouvet 1994, Taberlet et al. 1998).These studies hypothesize that populations were pushed into refugial ranges during the last glacial maxima. When glaciers receded, populations migrated back but were unable to traverse river systems inundated with glacial melt thus preventing genetic mixing.

Evidence of clear changes in the Mississippi River channel have been documented (Hudson and Kesel 2000). Due to the meandering nature of bottomland river systems, populations of deer may find themselves arbitrarily transferred across the river when a new river

channel is established, thus providing moments of genetic exchange. Additionally, myriad anecdotal evidence has shown that deer are capable of crossing the Mississippi River at its present day size (William McKinley, MDWFP, and Johnathan Bordelon, LDWF, *personal communication*). I documented evidence of this gene flow through clustering of Mississippi populations close to the river with western populations.

DeYoung et al. (2003) proposed a group of populations in Mississippi that were the result of natural regeneration of remnant native deer. These populations included Amite County, MS, Ashbrook, MS, Malmaison, MS, Noxubee WMA, MS, Sunflower, MS, Walker, MS, and Wilkinon, MS. My overall genetic assignment across all 73 populations showed some evidence of grouping between Amite County, MS, Walker, MS, East Mississippi Sportsman's Association, MS, and all of the Alabama populations. Genetic assignments across eastern southcentral populations revealed a similar grouping between Mississippi and Alabama deer. Over 65% of deer stocked in Alabama came from 3 counties that bordered Mississippi and were comprised of remnant native populations (Blackard 1971, McDonald and Miller 2004). My findings point to the evident success of these native stocks across Alabama and within portions of Mississippi as well.

I found some evidence of northern admixture within Union Parish, LA, Tensas NWR, LA and Black Warrior WMA, AL. However, the overall lack of genetic differentiation between northern and southcentral populations makes definitive conclusions difficult. Samples Wisconsin and Michigan were less differentiated from all southcentral populations (mean Nei's $D_A = 0.204$) than the average differentiation across southcentral populations (mean Nei's $D_A = 0.216$) or average differentiation between the western and eastern groups (mean Nei's $D_A = 0.224$). This is despite the fact that the average geographic distance between both Wisconsin and Michigan and

all southern populations was greater (1,494 km) than the average geographic distance between western and eastern groups (411 km). If genetic structure across the southeast U.S. has been influenced by Pleistocene glaciation, then gene flow north to south would be greater than gene flow west to east. Genetic assignment linking southcentral populations to Midwest deer may simply be an artifact of low genetic differentiation between those populations and not the presence of remnant northern genetics as a result of restoration efforts.

Indirect evidence shows that there is a high likelihood that northern stock sources did not fare well when translocated to the south. Lukefahr and Jacobson (1998) found that 65% of pure northern deer died upon exposure to EHD and Gaydos and colleages (2002) documented 100% mortality in fawns from Pennsylvania due to the same disease. Additionally, Blackard (1971) documents continued translocation efforts in locations that received northern stock sources, indicating a need for further restoration resulting from the original cohort of deer dying upon arrival. Northern stock likely did not survive long enough to have a lasting genetic impact on southern populations.

Translocation efforts to restore dwindling populations have resulted in detectable changes to genetic variation in a host of species including elk, turkeys, bears, and white-tailed deer (Brown et al. 2009, DeYoung et al. 2003, Hicks et al. 2007, Seidel et al. 2013). In the case of white-tailed deer, restocking occurred across large portions of their native range using a diverse array of source populations that did not account for local adaptation or genetic variation (Blackard 1971, McDonald and Miller 2004). These efforts have been largely heralded as a conservation success, with deer populations rebounding quickly and dramatically to their present day densities. Leberg (1990) warns about the use of non-native stock sources in translocation efforts, as they may result in deleterious genetic effects. It seems that through the happenstance

choice of easy-to-acquire native stock sources, state agencies in Louisiana, Mississippi, and Alabama may have mitigated these effects and maintained historic genetic variation within their deer herds.

MANAGEMENT IMPLICATIONS

The restoration of white-tailed deer during the 1900s has been heralded as one of the great conservation success stories. Deer populations rapidly rebounded and genetic diversity across the southeastern U.S. is high. However, evidence of restocking can still be seen in the high levels of admixture and the lack of any strong signal of isolation by distance. Additionally, it seems that the use of local native stock sources was widely successful in restoring populations while there is inconclusive evidence of success in using stocks from the Midwest, probably as a result of poor adaptation to the local environments in southern U.S. My findings highlight the importance in using stock sources that mirror the adaptive characteristics of the populations of concern. Additionally, I found ample evidence of the genetic effects of restocking on deer populations still present half a century after restoration efforts ended.

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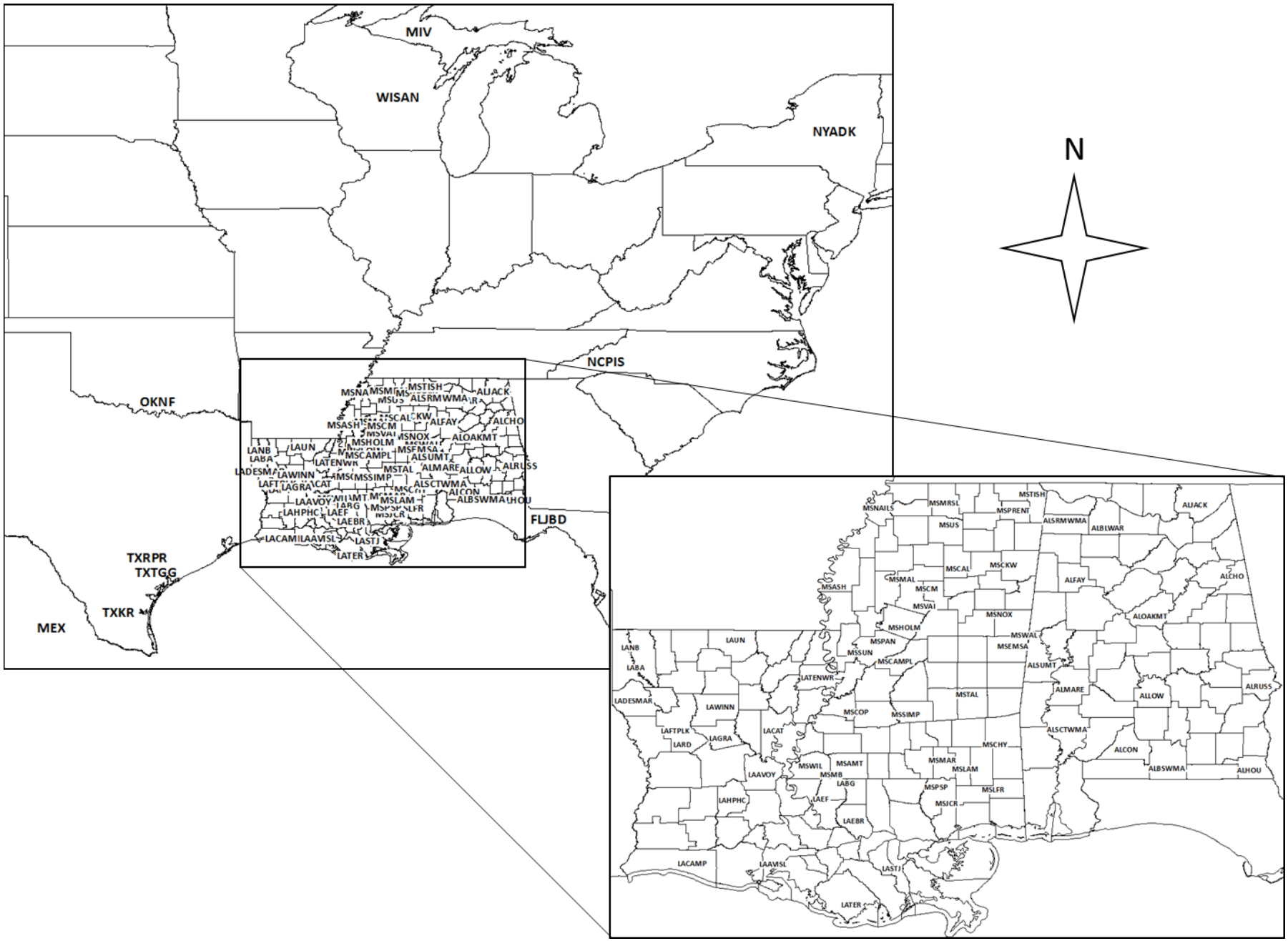
Table 1. List of southcentral U.S. sampling sites for genetic analysis of white-tailed deer. The translocation dates, number of deer stocked, and the stock source used is listed for each sample site that received restocking during the 1900s (Blackard 1971). Additionally, the purported lineage for each sample site is denoted by native (received only within-state stock sources), mixed (received both within and out-of-state stock sources), non-native (received only out-of-state stock sources, and N/A (received no stocking).

Population	Year Stocked	Total Deer	Stock Source	Lineage
Avery Island, LA	N/A	N/A	N/A	N/A
Avoyelles, LA	1969	34	Delta NWR, LA	Native
Barksdale, LA	1956-1957	66	Red Dirt, LA; Catahoula, LA	Native
Beechgrove, LA	Unk.	Unk.	Unk.	Unk.
Cameron, LA	1961-1969	83	Texas; Delta NWR, LA	Mixed
Camp Avondale, LA	1956, 1957, 1968	91	Red Dirt, LA; Catahoula, LA; Zemurray Park, LA; Delta NWR, LA	Native
Catahoula, LA	1956	31	Madison PAR; Tensas PAR	Native
DeSoto, LA	1955-1956	59	Red Dirt, LA; Catahoula, LA	Native
E. Baton Rouge, LA	1965	7	Gum Cove, LA	Native
Evangeline, LA	N/A	N/A	N/A	N/A
Grant, LA	1951-1956	95	Texas; Wisconsin; Red Dirt, LA	Mixed
Maurepas Swamp WMA, LA	1950-1951, 1969	62	Marsh Island, LA; Zemurray Park, LA; Avery Island, LA; Unk., LA; Delta NWR, LA; Wisconsin	Mixed
North Bossier, LA	1956-1957	66	Red Dirt, LA; Catahoula, LA	Native
Red Dirt, LA	Unk.	Unk.	Unk.	
Tensas NWR, LA	1952, 1966	31	Wisconsin	Non-native
Terrebone Parish, LA	1969	30	Delta NWR, LA	Native
Union, LA	1950-1959	118	Wisconsin; Madison PAR, LA; Tensas PAR, LA; Red Dirt, LA; Catahoula, LA	Mixed
Vernon, LA	1951-1963	147	Unk.; Avery Island, LA; Red Dirt, LA; Unk., LA; Madison PAR, LA; Zemurray Park, LA	Native
Winn, LA	1953	35	Madison PAR, LA; Tensas PAR, LA; Wisconsin	Mixed
Amite County, MS	1934-1939, 1959-1963	74	Adams County Refuge, MS; Yucatan Lake Island, MS; H.B. Cole Refuge, MS; Unk., MS Mexico	Mixed
Ashbrook, MS	1931, 1938	> 48	Louisiana; Mexico	Non-native
Calhoun, MS	Unk.	> 35	Leaf River, MS; Mexico	Mixed
Cameron Plantation, MS	1934-1939	3	Mexico	Non-native
Camp McCain, MS	1934-1939, 1952, 1956, 1958-1960, 1964	101	Leaf River, MS; Ran Baton, MS; Sardis Refuge, MS, Mexico	Mixed
Chickasaw, MS	1951-1955	74	Leaf River, MS; University Refuge, MS; Unk., MS	Native
Chickasawhay, MS	Unk.	57	Leaf River, MS; Mississippi; Wisconsin	Mixed
Copiah, MS	1953	20	Leaf River, MS	Mixed
East Mississippi Sportsman's Association, MS	Early 1930s	Unk.	Alabama	Non-native
Holmes, MS	1963	25	Sardis Refuge, MS	Mixed
Juniper Creek, MS	Unk.	> 176	Mississippi; Wisconsin	Mixed
Lamar County, MS	1957, 1959-1961, 1963, 1964	95	Ran Batson, MS; Unk., MS; Wisconsin	Mixed

Population	Year Stocked	Total Deer	Stock Source	Lineage
Leaf River, MS	1938-1943, 1948, 1962	> 74	Ran Batson, MS; Unk., MS; Mexico	Mixed
Malmaison WMA, MS	1934-1939, 1952, 1956, 1960	75	Mexico; Leaf River, MS; Ran Batson, MS; Sardis Refuge, MS	Mixed
Marion, MS	1951-1964	~365	H.B. Cole Refuge, MS; Unk., MS; Ran Batson, MS; Wisconsin, Leaf River, MS; Red Creek, MS	Mixed
Marshall, MS	1934-1960	~63	Mexico; Pisgah Game Preserve, NC; Sardis Refuge, MS	Mixed
Milbrook, MS	Unk.	Unk.	Unk.	Unk.
Nails Bayou Hunting Club, MS	1950-1957	31	Unk., MS; Leaf River, MS	Mixed
Noxubee NWR, MS	1947, 1952	44	Leaf River, MS; Kentucky	Mixed
Panther Swamp, MS	N/A	N/A	N/A	Native
Pine Springs, MS	Unk.	> 176	Mississippi; Mexico; Wisconsin	Mixed
Prentiss County, MS	1934-1952	8	Mexico, Unk., MS; Leaf River, MS	Mixed
Simpson County, MS	1934-1961	~80	Mexico, Unk., MS; Wisconsin; Leaf River, MS	Mixed
Sunflower, MS	N/A	N/A	N/A	Native
Tallahala, MS	1934-1939, 1945, 1948, 1949	> 112	Mexico; Leaf River, MS; Unk., MS;	Mixed
Tishomingo County, MS	1945-1949	~74	Leaf River, MS	Mixed
Upper Sardis, MS	1934-1939, 1949, 1953, 1960, 1961	> 109	Mexico; Leaf River, MS; Sardis Refuge, MS; Pisgah Game Preserve, NC	Mixed
Vaiden, MS	Unk.	> 113	Leaf River, MS; Mississippi; Wisconsin	Mixed
Walker, MS	Unk.	> 44	Leaf River, MS; Alabama; Kentucky	Mixed
Wilkinson, MS	N/A	N/A	N/A	Native
Black Warrior WMA, AL	1926-1964	142	Iron Mountain, MI; Clarke CO, AL; Marengo CO, AL	Mixed
Blue Springs WMA, AL	1951-1964	76	Texas, Clarke CO, AL	Mixed
Choccolocco WMA, AL	1939-1941	83	Pisgah Game Preserve, NC; Pets/Zoo, AL	Mixed
Conecuh, AL	N/A	N/A	N/A	N/A
Fayette, AL	1956-1962	8	Pets/Zoo, AL	Native
Houston, AL	N/A	N/A	N/A	N/A
Lowndes, AL	1958-1965	104	Clarke CO, AL; Marengo CO, AL	Native
Marengo, AL	N/A	N/A	N/A	Native
Oak Mountain State Park, AL	1946-1964	110	Pets/Zoo, AL; Clarke CO, AL; Sumter CO	Native
Russell, AL	N/A	N/A	N/A	N/A
Sam R. Murphy WMA, AL	1944-1965	148	Sumter CO, AL; Georgia; Clarke CO, AL; Marengo CO, AL	Mixed
Scotch WMA, AL	1926	12	Iron Mountain, MI; Washington CO, AL	Mixed
Skyline WMA, AL	1958-1963	111	Clarke CO, AL; Marengo CO, AL; Pets/Zoo, AL; Sumter CO, AL	Native
Sumter, AL	N/A	N/A	N/A	Native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LAFTPLK
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MSLAM
MSWIL
MSAMT
MSMB
LABG
MSPSP
MSLFR
MSICR

Figure 1. Populations of white-tailed deer sampled for genetic analysis across the United States and Mexico. Included are 10 outgroups for geographic scope and historical stock source representation as well as 63 southcentral U.S. populations (inset). Populations are: Mexico (MEX); King Ranch, TX (TXKR); Double G Ranch, TX (TXTGG); Runnels-Pierce Ranch, TX (TXRPR); Noble Foundation, OK (OKNF); Iron Mountain, MI (MIV); Sandhill WR, WI (WISAN); Adirondacks, NY (NYADK); Pisgah Game Preserve, NC (NCPIS); Joe Budd WMA, FL (FLJBD); North Bossier, LA (LANB); Barksdale, LA (LABA); Union Parish, LA (LAUN); Tensas NWR, LA (LATENWR); DeSoto Parish, LA (LADESMAR); Winn Parish, LA (LAWINN); Grant Parish, LA (LAGRA); Catahoula Parish, LA (LACAT); Vernon Parish, LA (LAFTPLK); Red Dirt, LA (LARD); Avoyelles Parish, LA (LAAVOY); Evangeline Parish, LA (LAHPHC); Cameron Parish, LA (LACAMP); Avery Island, LA (LAAVISL); Beechgrove, LA (LABG); Camp Avondale, LA (LAEF); E. Baton Rouge Parish, LA (LAEBR); Maurepas Swamp WMA, LA (LASTJ); Terrebone Parish, LA (LATER); Nails Bayou Hunting Club, MS (MSNAILS); Marshall County, MS (MNMRSL); Prentiss County, MS (MSPRENT); Tishomingo County, MS (MSTISH); Upper Sardis, MS (MSUS); Ashbrook, MS (MSASH); Malmaison WMA, MS (MSMAL); Camp McCain, MS (MSCM); Calhoun, MS (MSCAL); Chickasaw, MS (MSCKW); Vaiden, MS (MSVAI); Noxubee NWR, MS (MSNOX); Holmes County, MS (MSHOLM); Panther Swamp, MS (MSPAN); Sunflower, MS (MSSUN); Cameron Plantation, MS (MSCAMPL); Walker, MS (MSWAL); East Mississippi Sportsman's Association, MS (MSEMSA); Copiah County, MS (MSCOP); Simpson County, MS (MSSIMP); Tallahala, MS (MSTAL); Wilkinson, MS (MSWIL); Milbrook, MS (MSMB); Amite County, MS (MSAMT); Marion County, MS (MSMAR); Lamar County, MS (MSLAM); Chickasawhay, MS (MSCHY); Pine Springs, MS (MSPSP); Leaf River, MS (MSLFR); Juniper Creek, MS (MSJCR); Sam R. Murphy WMA, AL (ALSRMWMA); Black Warrior WMA, AL (ALBLWAR); Skyline WMA, AL (ALJACK); Fayette County, AL (ALFAY); Oak Mountain State Park, AL (ALOAKMT); Choccolocco WMA, AL (ALCHO); Sumter County, AL (ALSUMT); Marengo County, AL (ALMARE); Lowndes County, AL (ALLOW); Russell County, AL (ALRUSS); Scotch WMA, AL (ALSCTWMA); Conecuh County, AL (ALCON); Blue Springs WMA, AL (ALBSWMA); and Houston County, AL (ALHOU).

Table 2. Summary statistics of genetic diversity including sample size (*N*), average inbreeding coefficient (F_{IS}) with standard deviation, average gene diversity (*H*) with standard deviation, and significant pairwise linkage disequilibrium (*LD*) for all 63 populations of white-tailed deer analyzed across southcentral U.S. and 10 geographic outgroups.

Population	*N*	F_{IS} (SD)	*H* (SD)	HWE	*LD*
Adirondacks, NY	30	0.05 (0.11)	0.77 (0.15)	14	0
Double G Ranch, TX	33	0.07 (0.16)	0.74 (0.16)	14	8
Iron Mountain, MI	30	0.03 (0.13)	0.78 (0.17)	14	2
Joe Budd, FL	30	0.13 (0.16)	0.74 (0.16)	14	5
King Ranch, TX	39	0.03 (0.16)	0.76 (0.12)	14	5
Mexico	37	0.05 (0.12)	0.77 (0.14)	14	2
Noble Foundation, OK	30	0.07 (0.16)	0.75 (0.14)	14	2
Pisgah Game Preserve, NC	26	0.16 (0.12)	0.70 (0.16)	14	4
Runnels-Pierce Ranch, TX	10	0.06 (0.23)	0.60 (0.30)	12	2
Sandhill WR, WI	26	0.03 (0.13)	0.74 (0.18)	14	1
Avery Island, LA	29	0.07 (0.12)	0.72 (0.19)	14	1
Avoyelles Parish, LA	30	0.08 (0.18)	0.74 (0.19)	14	3
Barksdale, LA	48	0.14 (0.11)	0.78 (0.14)	14	6
Beechgrove, LA	39	0.06 (0.12)	0.79 (0.15)	14	5
Cameron Parish, LA	6	-0.02 (0.20)	0.67 (0.18)	14	0
Camp Avondale, LA	35	0.07 (0.13)	0.76 (0.18)	14	3
Catahoula Parish, LA	23	0.02 (0.16)	0.72 (0.22)	13	6
Desoto Parish, LA	30	0.08 (0.09)	0.75 (0.15)	14	3
East Baton Rouge Parish, LA	39	0.06 (0.11)	0.74 (0.20)	14	1
Evangeline Parish, LA	31	0.06 (0.10)	0.79 (0.10)	14	3
Fort Polk, LA	30	0.06 (0.13)	0.78 (0.13)	14	2
Grant Parish, LA	30	0.14 (0.21)	0.71 (0.18)	14	3
Maurepas Swamp WMA, LA	24	0.09 (0.18)	0.76 (0.17)	14	3
North Bossier, LA	24	0.13 (0.16)	0.76 (0.14)	14	1
Red Dirt, LA	11	-0.05 (0.16)	0.73 (0.23)	13	0
Tensas NWR, LA	30	0.02 (0.13)	0.74 (0.19)	14	0
Terrebone Parish, LA	41	0.07 (0.11)	0.74 (0.13)	14	3
Union Parish, LA	22	0.01 (0.15)	0.78 (0.13)	14	0
Winn Parish, LA	20	0.03 (0.17)	0.74 (0.18)	14	2
Amite County, MS	42	0.06 (0.11)	0.78 (0.15)	14	0
Ashbrook, MS	29	0.05 (0.09)	0.75 (0.20)	14	1
Calhoun, MS	27	0.07 (0.12)	0.77 (0.14)	14	3
Cameron Plantation, MS	34	0.08 (0.14)	0.78 (0.18)	14	4

Population	N	FIS (SD)	H (SD)	HWE	LD
Camp McCain, MS	27	-0.04 (0.13)	0.76 (0.12)	14	1
Chickasaw, MS	21	0.00 (0.13)	0.78 (0.12)	14	1
Chickasawhay, MS	24	0.09 (0.16)	0.73 (0.18)	14	6
Copiah County, MS	34	0.07 (0.12)	0.74 (0.21)	14	6
Eastern Mississippi Sportsman's Association, MS	31	0.01 (0.06)	0.74 (0.14)	14	2
Holmes County, MS	33	0.11 (0.13)	0.77 (0.16)	14	1
Juniper Creek, MS	24	0.06 (0.12)	0.68 (0.24)	14	9
Lamar County, MS	19	0.05 (0.12)	0.76 (0.22)	14	3
Leaf River, MS	38	0.01 (0.10)	0.73 (0.19)	14	2
Malmaison WMA, MS	20	0.08 (0.13)	0.74 (0.11)	14	2
Marion County, MS	23	0.06 (0.14)	0.74 (0.23)	14	1
Marshall County, MS	16	0.15 (0.18)	0.72 (0.22)	14	2
Milbrook, MS	22	0.05 (0.13)	0.76 (0.19)	14	0
Nails Bayou Hunting Club, MS	14	0.02 (0.21)	0.74 (0.17)	14	1
Noxubee NWR, MS	40	0.10 (0.11)	0.78 (0.12)	14	2
Panther Swamp, MS	18	0.14 (0.21)	0.75 (0.17)	14	6
Pine Springs, MS	29	0.09 (0.13)	0.75 (0.18)	14	0
Prentiss County, MS	19	0.05 (0.14)	0.77 (0.17)	14	0
Simpson County, MS	17	0.01 (0.11)	0.77 (0.11)	14	1
Sunflower, MS	20	0.04 (0.14)	0.74 (0.20)	14	2
Tallahala, MS	33	0.05 (0.12)	0.74 (0.17)	14	3
Tishomingo County, MS	23	0.06 (0.14)	0.75 (0.19)	14	0
Upper Sardis, MS	31	0.09 (0.21)	0.73 (0.15)	14	1
Vaiden, MS	32	0.03 (0.08)	0.78 (0.14)	14	1
Walker, MS	16	0.03 (0.22)	0.70 (0.13)	14	1
Wilkinson, MS	22	0.04 (0.16)	0.75 (0.20)	14	1
Black Warrior WMA, AL	30	0.07 (0.12)	0.76 (0.13)	14	0
Blue Springs WMA, AL	30	0.04 (0.11)	0.78 (0.14)	14	0
Choccolocco WMA, AL	16	-0.04 (0.18)	0.73 (0.16)	14	0
Conecuh County, AL	28	0.10 (0.14)	0.73 (0.19)	14	1
Fayette County, AL	29	0.04 (0.17)	0.72 (0.18)	14	1
Houston County, AL	29	0.07 (0.10)	0.78 (0.14)	14	0
Lowndes County, AL	30	0.08 (0.11)	0.76 (0.17)	14	0
Marengo County, AL	27	0.08 (0.14)	0.76 (0.16)	14	1
Oak Mountain State Park, AL	31	0.07 (0.16)	0.74 (0.16)	14	2
Russell County, AL	39	0.14 (0.15)	0.77 (0.13)	14	7
Sam R. Murphy WMA, AL	30	0.00 (0.11)	0.75 (0.16)	14	1
Scotch WMA, AL	30	0.06 (0.13)	0.75 (0.16)	14	0
Skyline WMA, AL	30	0.11 (0.14)	0.73 (0.19)	14	4
Sumter County, AL	24	0.12 (0.19)	0.74 (0.15)	14	0

Table 3. Analysis of Molecular Variance (AMOVA) comparing 2 groups of white-tailed deer comprised of all populations west of the Mississippi River and all populations east of the Mississippi River without northern populations included (a) and groups comprised of the Wisconsin and Michigan populations and all the southcentral populations (b).

a)

Source of variation	Sum of squares	Variance components	Percentage variation
Among west and east groups	91.2	<0.1	0.9
Among populations within west and east groups	1149.1	0.2	4.9
Among individuals within populations	8486.7	0.3	5.9
Within individuals	7784.5	4.2	88.3
Total	17511.5	4.7	

b)

Source of variation	Sum of squares	Variance components	Percentage variation
Among Midwestern and southern groups	18.9	0.0	<0.1
Among populations within Midwestern and southern groups	246.6	0.2	4.5
Among individuals within populations	2113.5	0.4	6.4
Within individuals	1931.5	4.9	89.2
Total	4310.6	5.5	

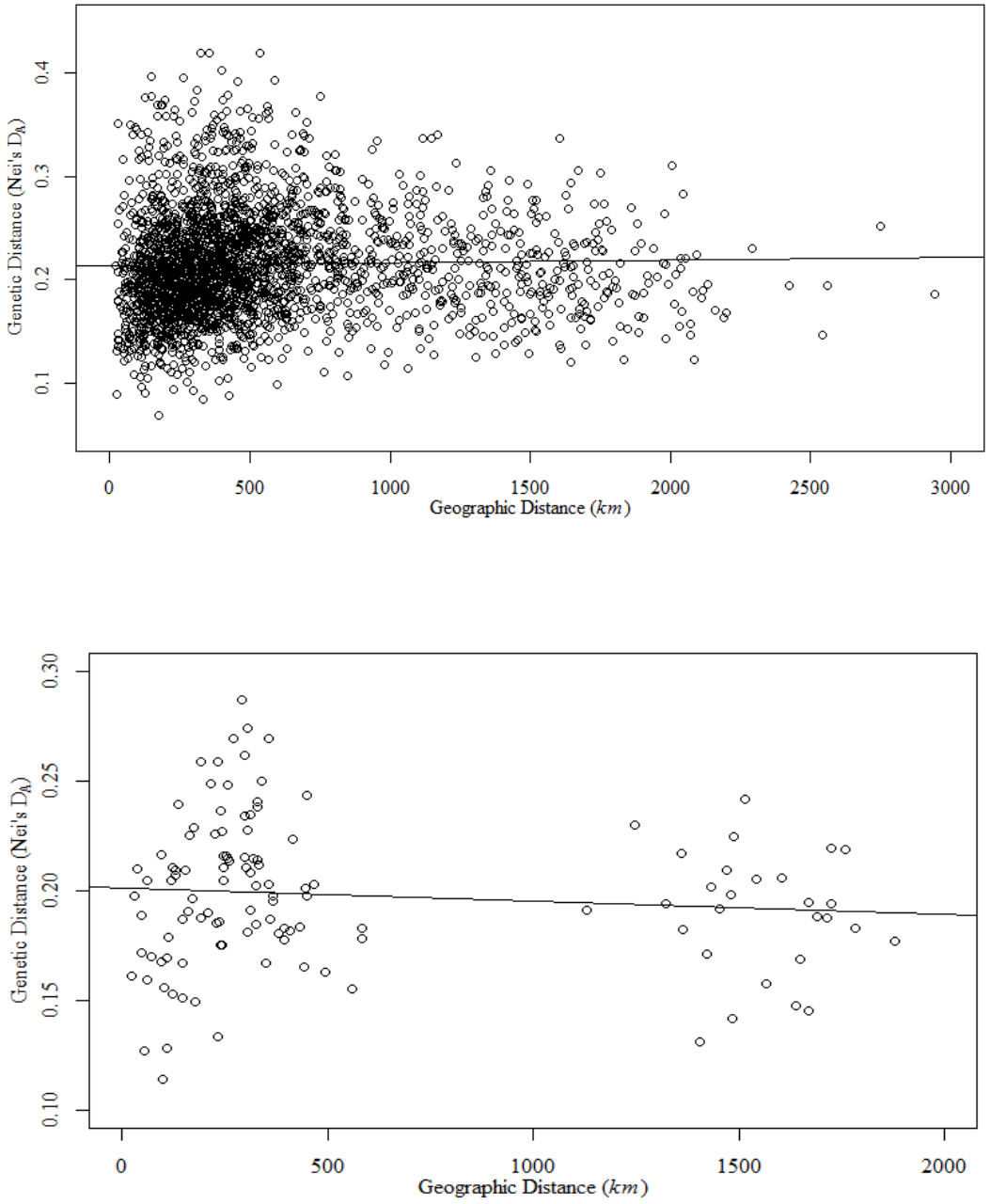

Figure 2. Relationship between genetic distance (Nei's D_A) and geographic distance (Km) for all 73 populations of white-tailed deer (P=0.722, R^2 = 0.001, slope = 2.9×10^{-6}; top) and between southcentral populations that received northern stock sources and Midwestern populations (P = 0.612, R^2 = 0.011, slope = -6.2×10^{-6}; bottom).

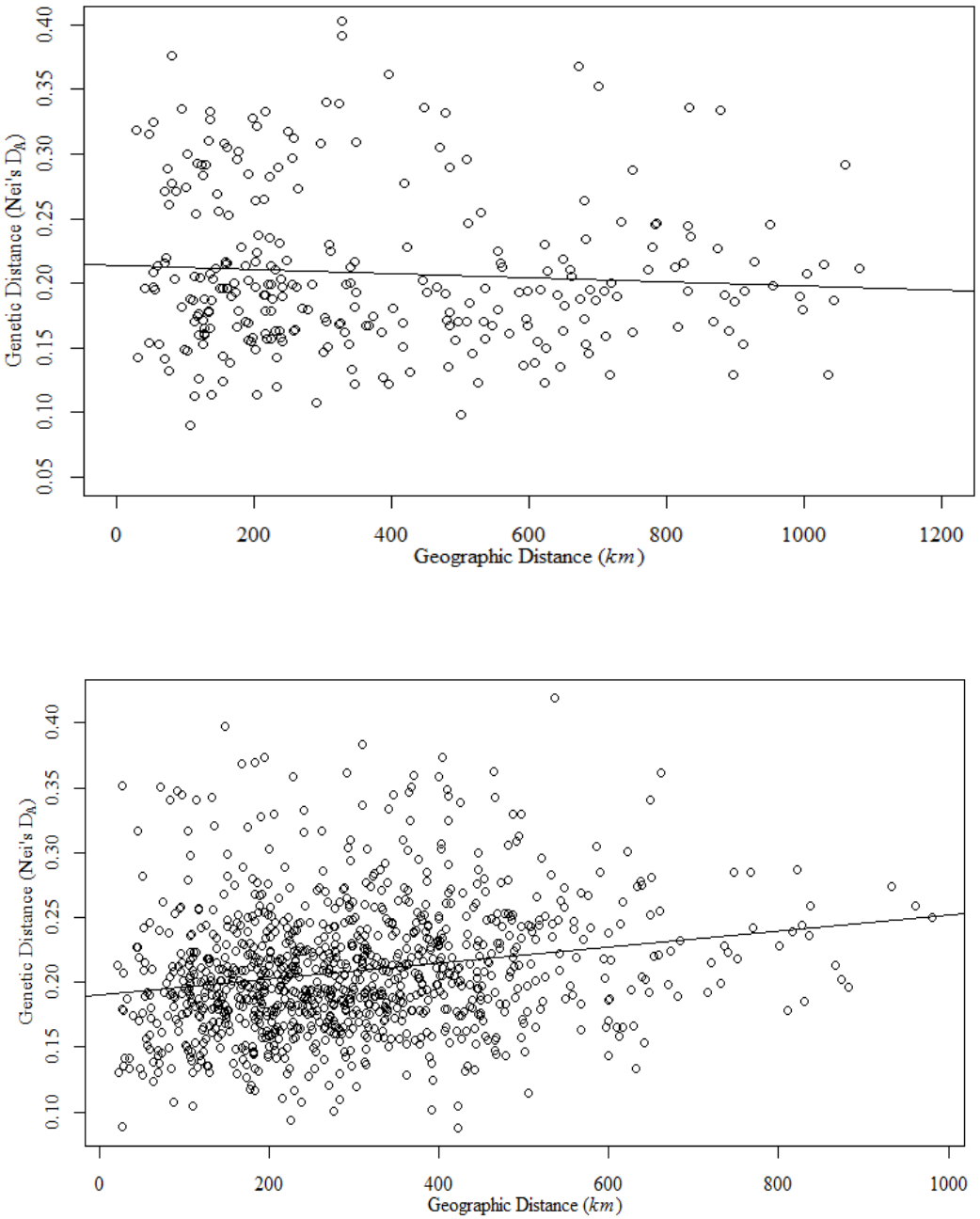

Figure 3. Relationship between genetic distance (Nei's D_A) and geographic distance (Km) for populations of white-tailed deer west of the Mississippi River (P = 0.771, R^2 = 0.014, slope = -2.7x10^{-5}; top) and populations east of the Mississippi River (P = 0.001, R^2 = 0.090, slope = 7.7x10^{-5}; bottom).

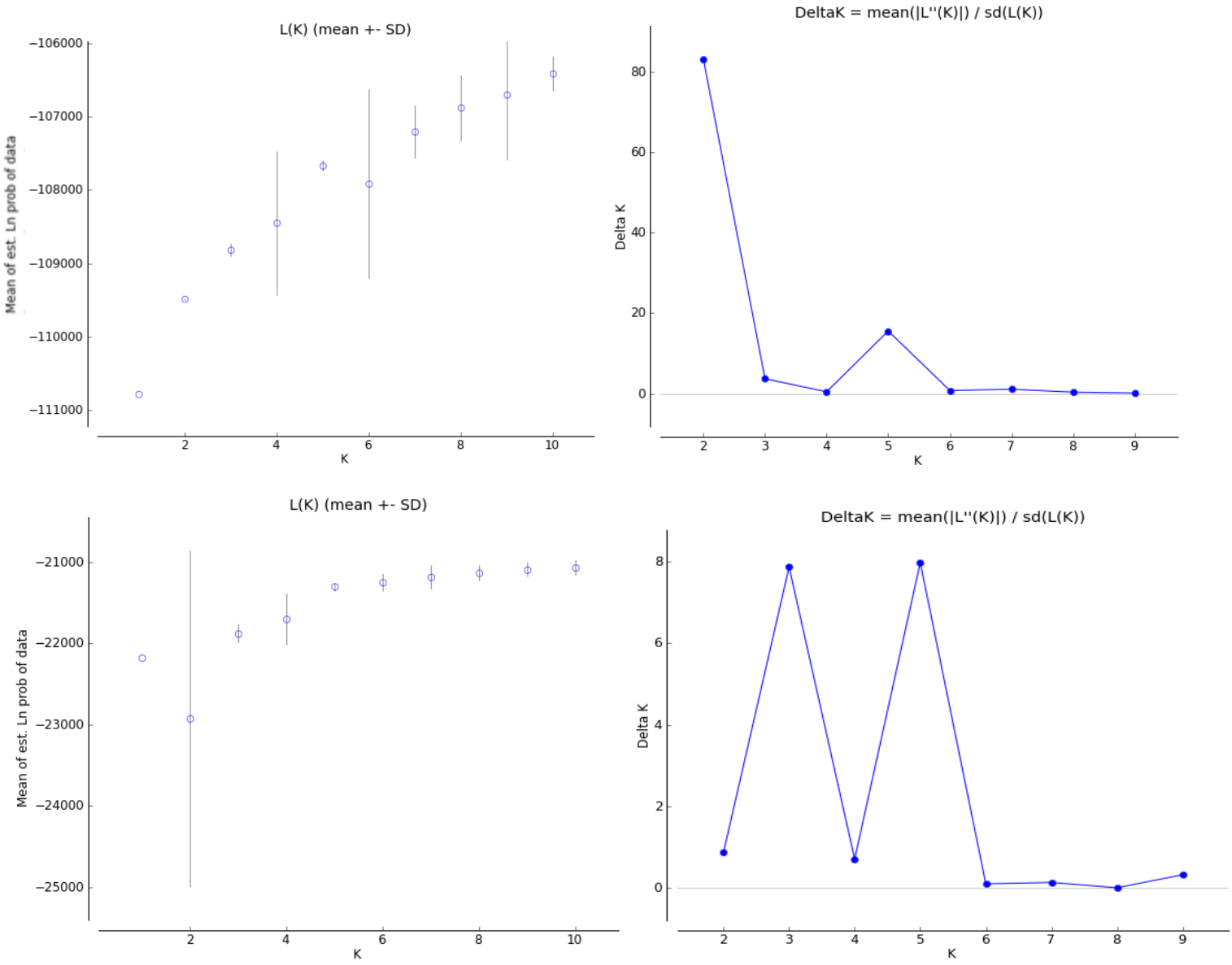

Figure 4. Pritchard et al. 2000's average estimated natural logarithm of the probability of K (Ln Pr(X | K), left) and Evanno et al. 2005's comparison of the second order rate of change (ΔK) to the number of prospective groups (right) for all 73 populations of white-tailed deer (top) and for southcentral populations that received northern stock sources (bottom).

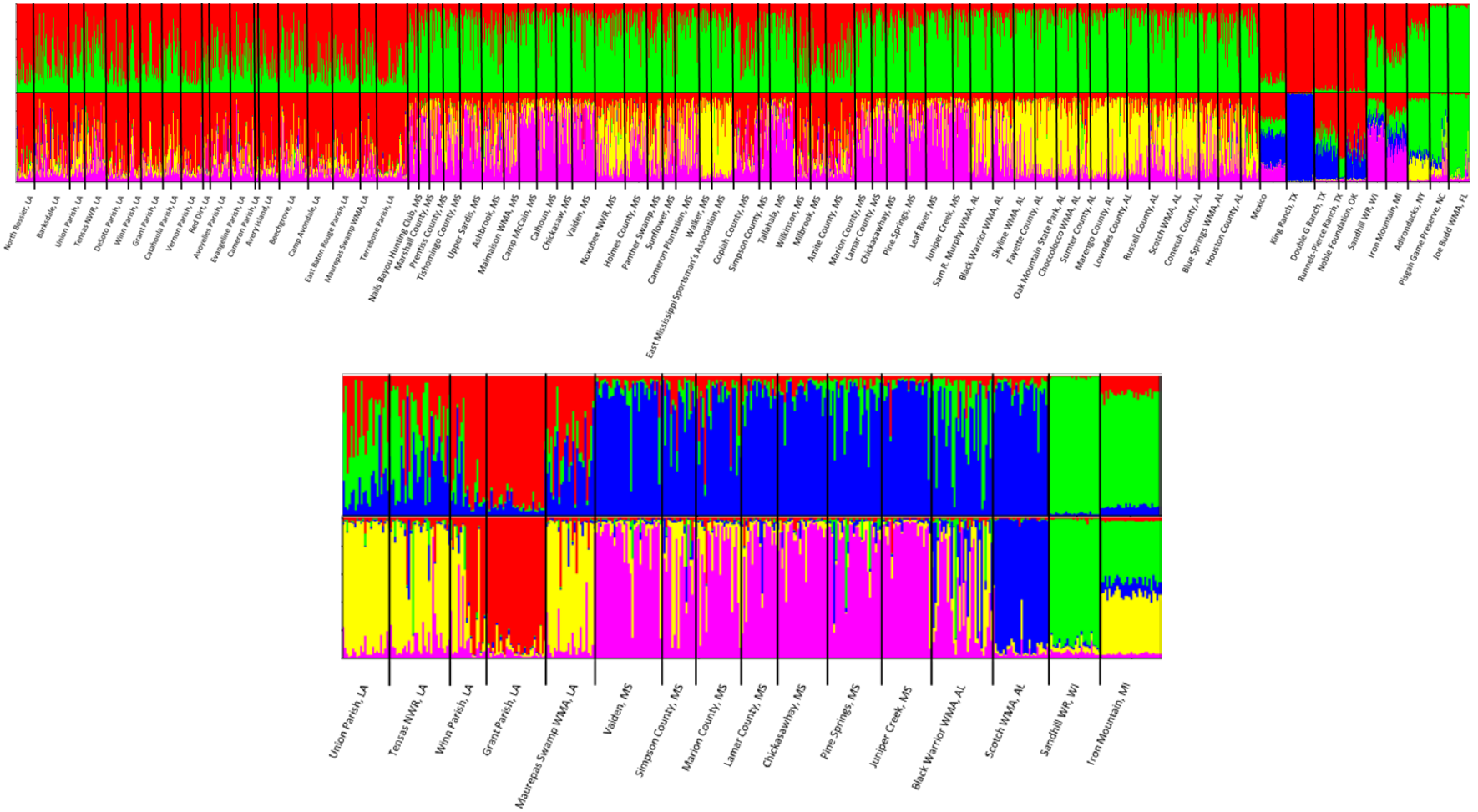

Figure 5. STRUCTURE output all 63 southcentral white-tailed deer populations compared to 10 outgroup populations at K=2 and K=5 (top) and comparisons of southcentral populations that had received northern stock sources with Wisconsin and Michigan populations at K=3 and K=5 (bottom). Both comparisons used the LOCPRIOR model by designating outgroup populations as a prior to inform assignment of southcentral individuals.

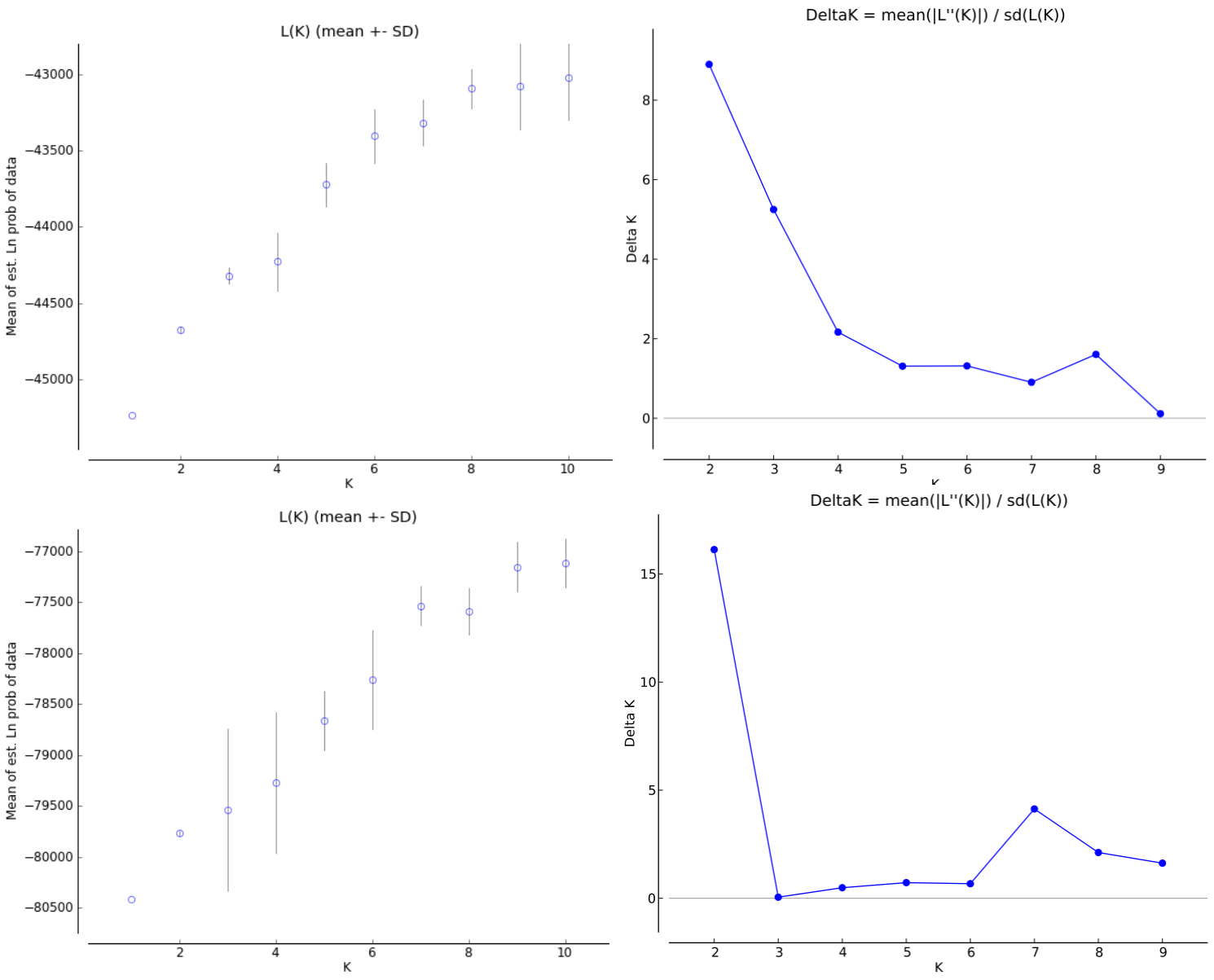

Figure 6. Pritchard et al. 2000's average estimated natural logarithm of the probability of K (Ln Pr(X | K), left) and Evanno et al. 2005's comparison of the second order rate of change (ΔK) to the number of prospective groups (right) for all white-tailed deer populations west of the Mississippi River (top) and for all populations to the east of the Mississippi River (bottom).

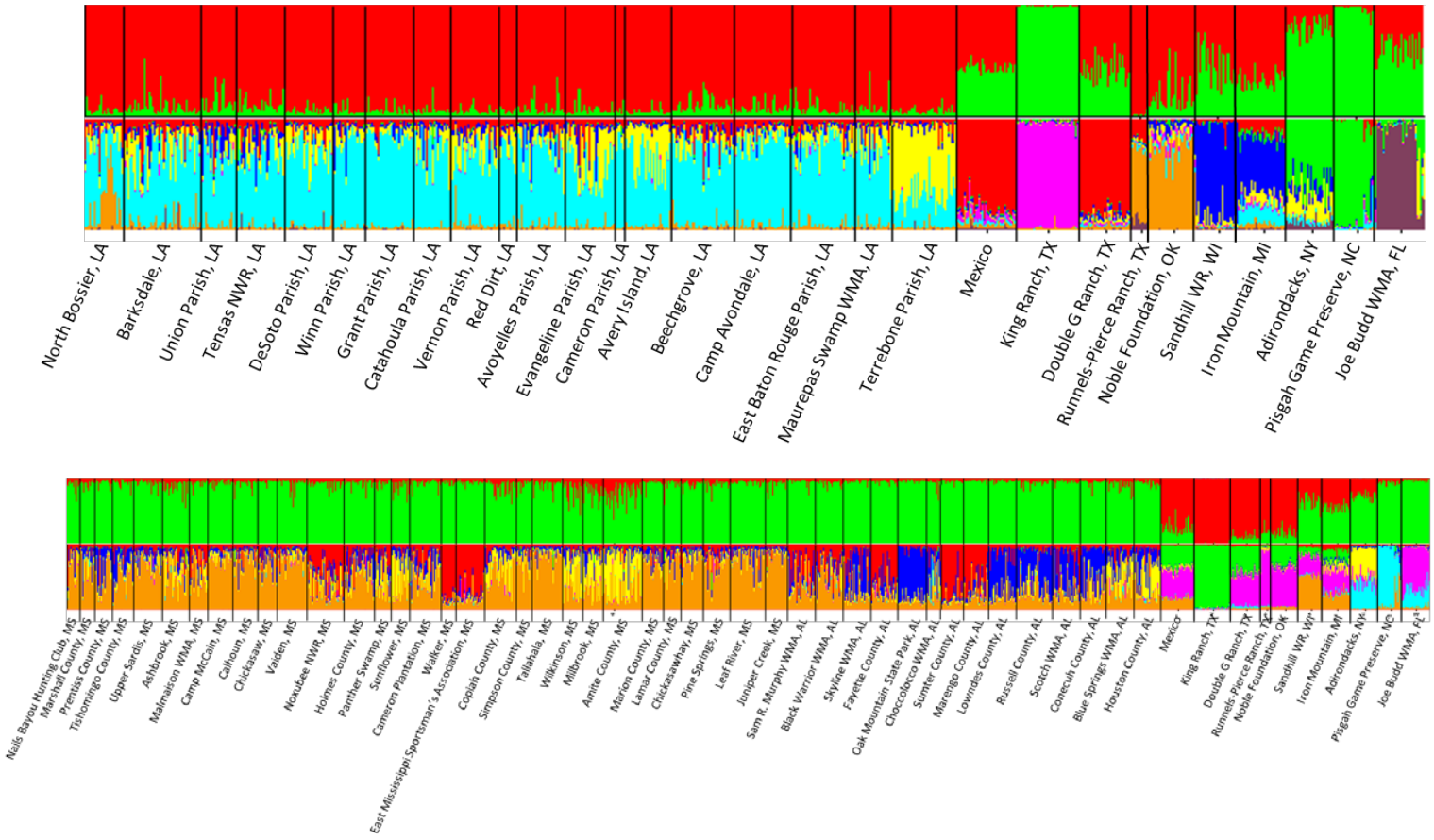

Figure 7. STRUCTURE results showing all western-southcentral populations of white-tailed deer compared to outgroup populations at clustering groups of K=2 and K=8 (top) and all east-southcentral populations of white-tailed deer compared to outgroup populations at K-2 and K=7 (bottom).

CHAPTER 2

EVALUATION OF MICROSATELLITE MARKERS TO DIFFERENTIATE MULTI-GENERATIONAL OFFSPRING OF PEN-RAISED DEER IN A FREE-RANGING DEER POPULATION

INTRODUCTION

Populations of white-tailed deer have been mixed during the last century due to continued manipulation for conservation and management purposes in the United States. This process began during the early 1900s when deer populations had dwindled due to unregulated hunting and habitat loss. State agencies began to restore native populations of southern deer by introducing stocks sources from across North America (Blackard 1971, McDonald et al 2004). The modern genetic consequences are evidenced by high levels of admixture in restored populations (Karlin et al 1989, Leberg et al 1994, Leberg and Ellsworth 1999, DeYoung et al 2003b, Sumners et al 2015). Additionally, there is the question of whether non-native genetic variation remains, particularly from northern stock sources used during the restoration. The result is a free-range population of deer that has been heavily influenced by human intervention.

Recently, the propagation and sale of captive deer has become a large industry that totals more than 10,000 facilities (Adams et al. 2014). Specifically, this industry uses animal husbandry methods and line-breeding to produce large-antlered deer for use in fenced-hunting operations and as breeding stock for sale. To this end, the deer breeding industry has facilitated the shipment of live deer along with sperm and ova across state lines throughout the country (Sabalow 2014). Although deer breeding facilities are required to prevent egress, the purposeful mixing of native and non-native pedigrees within breeding pens and high-fence enclosures further complicates the analysis of local genetic structure.

This introgression of non-native, captive lineages into free-range populations represents a commonly occurring front of hybridization in the modern world (Allendorf et al 2001, Randi 2008). Pedigrees used in deer breeding facilities in the southeastern United States often come from regions of the United States originally used as source stock for restoration efforts in the 1900s, such as the Midwest and Texas (Blackard 1971). This results in two waves of introgressive hybridization between native and non-native populations – first during restocking and second during animal husbandry practices in breeding pens. In certain cases, this introgression may result in hybrid swarms wherein F1 hybrids backcross with free-range or genetically manipulated populations (Allendorf et al. 2001). This has greatly complicated the investigation of illegal transfers or releases of deer as well as tracking the spread of diseases such as chronic wasting disease (CWD).

The designation of parent, F1, and F2 generations within breeding facilities and wild deer populations has the potential to be a valuable tool for white-tailed deer management, especially as state agencies attempt to better understand local genetic variation. Such knowledge will allow state agencies to better detect illegal shipments of deer, escaped individuals from breeding pens, and determine the source population of CWD-positive deer. Additionally, the ability to test pedigrees will allow states to consider deactivation clauses, where breeding facilities and high-fence enclosures may remove fences if they can prove the absence of genetically manipulated lineages. Finally, this tool will allow breeding pen owners to determine if release of captive deer into high-fence enclosures measurably changes the genetic variation within the enclosure population. However, the ability to identify genetically-manipulated individuals diminishes with subsequent generations of introgression. Therefore, our ability to correctly assign F1 and F2 hybrids in admixed populations of free-range and captive deer needs to be more fully studied.

The objective of my study was to estimate genetic differentiation between captive and free-ranging stocks and evaluate the ability to detect admixture between captive and free-ranging stocks. I used empirical data and simulations based on empirical data from captive and free-ranging deer in the southeastern US to assess the efficacy of microsatellite markers to differentiate first and subsequent generation offspring of pen-raised deer in native populations. In cases where genetically manipulated lineages have mixed with free-ranging populations of deer, the ability to predict resulting F1 and F2 offspring may prove a valuable tool in the delineation of novel pedigrees.

STUDY AREA

Captive populations

Deer can be enclosed at two scales: high fence enclosures and breeding pens. High fence enclosures are properties in which a fence (generally mesh, 2.4 m tall) prevents ingress and egress of deer. While managers may manipulate the genetic structure of this population through the physical transportation of new deer into the facility, typically they use traditional methods of selective harvest. Alternatively, breeding pens are fenced enclosures erected within a high-fence facility. Controlled breeding within these pens, including natural line breeding and artificial insemination, designed to increase the probability of offspring with larger antlers will likely alter the genetic characteristics of the subsequent population. In many states, artificial insemination with imported semen is the only legal way to introduce non-native pedigrees, due to restrictions on the importation of live animals. Offspring produced in breeding pens are often released into a larger high-fence enclosure for harvest, or with the purpose of propagating their manipulated pedigrees. Demarais et al. (2016) demonstrated that release of breeding pen deer into an enclosure population can have a measurable effect on antler size given a sufficient replacement

rate. As such, high fence enclosures that house a breeding pen may contain a mixture of genetically manipulated deer and deer genetically similar to the adjacent free-ranging population if a sufficient number of animals had been released.

I assessed 6 breeding pens across the southcentral United States. Breeding pens were located in Louisiana (LA-1), Mississippi (MS-1, MS-2, MS-3, and MS-4), and Alabama (AL-1). For 3 of these facilities (MS-1, MS-2, and MS-3), I was also able to obtain samples from adjacent high-fenced enclosures where deer from the breeding pen deer were released to co-mingle with wild deer managed within the enclosures. The wild deer were presumed to be local stocks enclosed during construction of the game-proof fence. Two of the Mississippi facilities, MS-1 and MS-2, were shut down due to the illegal transportation of white-tailed deer across state borders in violation of the Lacey Act. Mississippi Department of Wildlife, Fisheries, and Parks (MDWFP) biologists depopulated these two breeding pens and their surrounding high-fenced enclosures and sampled each deer. I sampled the 2 additional Mississippi breeding pens during Fall 2015. One of these breeding pens also had a surrounding enclosure where breeding pen individuals were released each year. We acquired samples out of this enclosure from hunter-harvested deer taken during the 2015-2016 hunting season. Owners of the Louisiana and Alabama breeding pens submitted hair samples from penned deer.

Free-range populations

I sampled the wild, free-ranging deer populations immediately around each of the breeding facilities. The free-ranging populations served as the baseline genetic control for our analysis. I assumed that geographically-proximate free-range deer populations would be the most direct comparison to genetically-manipulated deer within the breeding pens. Free-range samples for

one population used in comparisons with MS-1 and one population used in comparisons with MS-2 came from archived data from DeYoung et al. (2003) collected during 1998-1999.

METHODS

DNA Sample Collection

Captive populations

Myself or state biologists collected either tissue or hair samples from breeding pens and enclosures from 2015-2017. I placed each hair sample from breeding pens in a labeled manila envelope, and stored samples at Mississippi State University inside sealed plastic bags containing desiccant packets. Agency biologists stored tissue samples in sealed plastic bags and transferred on ice to Mississippi State University to be stored at -20ºC. I stored extracted DNA from both hair and tissue samples at -80ºC.

Free-range populations

State agency employees or their agents sampled free-range populations by collecting the end 2-cm of tongues from hunter-harvested deer during the 2015-16 and 2016-17 deer hunting seasons excepting archived samples from DeYoung et al. (2003a). Each sample was placed into a labeled bag and frozen. I placed stored samples at -20ºC until DNA extraction. I then stored extracted samples at -80ºC.

DNA Extraction and Amplification

I extracted DNA using the Qiagen® DNeasy™ Tissue Kit (QIAGEN Genomics Inc., Hilden, Germany), following the manufacturer's protocol. I used a 15-locus microsatellite panel, including BL25, BM4208, BM6438, BM6506, BM848, Cervid1, ILSTS011, INRA011, and OarFCB193, D, K, N, O, P, and Q (Anderson et al. 2002, DeYoung et al. 2003b). I amplified loci in 4 multiplex reactions, as described by Anderson et al. (2002) and DeYoung et al. (2003a),

and loaded the resulting products onto an automated genetic analyzer for separation and detection (3130*xl*, Applied Biosystems, Foster City, CA). I determined allele size calls for each locus using GeneMapper 4.0 (Applied Biosystems).

Genetic data from DeYoung et al. (2003) was collected on a different sequencing platform than the one I used. To ensure that differences in migration of fragments did not affect allele size calls, I re-amplified and genotyped 71 archived DNA samples from DeYoung et al. (2003) and Sumners et al. (2015), chosen to be representative of allele frequencies across those populations. I used those genotypes to calibrate bins to ensure microsatellite allele calls matched between datasets, but was unable to accurately assign allele calls for the Q locus; therefore I omitted that locus from further analyses.

DNA Analysis

Hybrid Analysis

To assess my ability to differentiate F1 and back-cross generations from their parent populations, I simulated mating following the STRUCTURE-training approach outlined in Latch et al. (2011). I assessed simulated F1 and back-crosses for the 6 breeding pens and their adjacent free-range populations. I first subset parental generations based on their admixture coefficients. I derived these admixture coefficients from an initial STRUCTURE run using just the parental populations (breeding pen and free-range) by running STRUCTURE at K = 2 for a burn-in of 100,000 followed by 500,000 permutations using the admixture and correlated allele frequency models. I then subset the parental populations by choosing only individuals with coefficients over 90%. I used these subset parental populations to simulate my hybrid crosses between captive and wild populations.

I conducted pairwise F_{ST} between each paired breeding pen and free-range population to assess the level of differentiation between parental populations. I used the package HIERFSTAT (Goudet et al. 2017) in Program R (R Core Team 2015) to generate Nei's F_{ST} (Nei 1973). I limited the populations to subset training parental populations to determine how different populations of breeding pen and free-range deer were from each other.

I then simulated hybridization to create 2,000 F1 individuals (breeding pen × free-range), 2,000 F2-free-range individuals (F1 × free-range), and 2,000 F2-breeding pen individuals (F1 × breeding pen). These crosses provide a continuum of genetic differentiation wherein my ability to detect differences would fade from parent populations versus F1 towards parent populations versus F2. I combined the simulated hybrids and exported them as a STRUCTURE file. I then conducted STRUCTURE run with a 100,000 MCMC burn-in, followed by 500,000 permutations at a K-value of 2 using the admixture, correlated allele frequency models. I also designated each generation as a separate population using the prior USEPOPINFO. I then used the resulting ancestry proportions to develop 90%-confidence intervals to determine whether distributions of each generation overlapped.

Comparisons Between Breeding Pens, Enclosures, and Free-range Populations

I used the 3 properties with breeding pen, enclosure, and free-range populations to assess introgression of breeding pen stock into a native population. I used 2 slightly different methods to conduct assignment tests through STRUCTURE. In both methods, I used the training dataset of pure breeding pen and free-range individuals to compare to the unknown enclosure deer. However, in the first method, called the "USEPOPINFO Method", I designated each of the 3 populations for each comparison (breeding pen, free-range, and enclosure) using the hard prior of USEPOPINFO in STRUCTURE. For the second method, called the "LOCPRIOR Method," I

designated the breeding pen and free-range populations as known populations using the LOCPRIOR model in STRUCTURE. The enclosure population was modeled as unknown so that STRUCTURE would use allele frequencies from the known populations to inform the assignment of unknown individuals. I ran a 100,000 MCMC burn-in followed by 500,000 permutations at a K-value of 2 using the admixture, correlated allele frequency models for both methods. I then categorized individuals into the 90% confidence-intervals described in the hybrid analysis.

RESULTS

Hybrid Analysis

For the Louisiana site, I started with 33 breeding pen individuals and 53 free-range individuals from 2 populations. Using this as my training dataset of known breeding pen and free-range individuals, I removed 4 breeding pen individuals and 9 free-range individuals based on their q-values. I simulated hybridization between 29 breeding pen individuals and 44 free-range individuals; pairwise F_{ST} between the 2 stocks was 0.045 (Table 1). I only used 13 loci for this simulation because the free-range population was missing data from the N locus. Average ancestry proportions for each parental and hybrid generation ranged from 0.222 to 0.769 (Fig. 1). The 90% confidence intervals revealed overlaps between F2-backcrosses and both F1s and the respective parental population. However, parental populations were differentiated from each other and from the F1 hybrids (Fig. 1).

For the MS-1 site, I started with 19 breeding pen individuals and 29 free-range individuals. Using this as my training dataset of known breeding pen and free-range individuals, I removed 5 breeding pen individuals based on their q-values and simulated hybridization between 14 breeding pen individuals and 29 free-range individuals. Pairwise F_{ST} was 0.050

between the 2 stocks (Table 1). I also only used 13 loci for my simulation; the MS-1 was missing data for the ILSTS locus and, therefore, I removed it from the analysis. Average ancestry proportions for each parental and hybrid generation ranged from 0.148 to 0.801 (Fig. 1). The 90% confidence intervals revealed overlaps between F2-backcrosses and both F1s and the respective parental population. However, parental populations were differentiated from each other and from the F1 hybrids (Fig. 1).

For the MS-2 site, I started with 23 breeding pen individuals and 48 free-range individuals from 2 populations. Using this as my training dataset of known breeding pen and free-range individuals, I removed 5 breeding pen individuals and 8 free-range individuals based on their q-values. I simulated hybridization between 18 breeding pen individuals and 40 free-range individuals; pairwise F_{ST} between stocks was 0.036 (Table 1). I also only used 13 loci for my simulation because most MS-2 individuals were missing data for the ILSTS locus. Average ancestry proportions for each parental and hybrid generation ranged from 0.155 to 0.826 (Fig. 1). The 90% confidence intervals revealed overlaps between F2-backcrosses and both F1s and the respective parental population. However, parental populations were differentiated from each other and from the F1 hybrids (Fig. 1).

For the MS-3 site, I started with 82 breeding pen individuals and 30 free-range individuals. Using this as my training dataset of known breeding pen and free-range individuals, I removed 61 breeding pen individuals and 6 free-range individuals based on their q-values. I simulated hybridization between 21 breeding pen individuals and 24 free-range individuals; pairwise F_{ST} between stocks was 0.068 (Table 1). Average ancestry proportions for each parental and hybrid generation ranged from 0.163 to 0.833 (Figure 1). The 90% confidence intervals revealed overlaps between F2-backcrosses and both F1s and the respective parental population.

However, parental populations were differentiated from each other and from the F1 hybrids (Fig. 1).

For the MS-4 site, I started with 105 breeding pen individuals and 48 free-range individuals from 2 populations. Using this as my training dataset of known breeding pen and free-range individuals, I removed 60 breeding pen individuals and 14 free-range individuals based on their q-values. I then simulated hybridization between 45 breeding pen individuals and 34 free-range individuals; pairwise F_{ST} between stocks was 0.060 (Table 1). Average ancestry proportions for each parental and hybrid generation ranged from 0.164 to 0.815 (Fig. 1). The 90% confidence intervals revealed overlaps between F2-backcrosses and both F1s and the respective parental population. However, parental populations were differentiated from each other and from the F1 hybrids (Fig. 1).

For the Alabama site, I started with 53 breeding pen individuals and 58 free-range individuals from 2 populations. Using this as my training dataset of known breeding pen and free-range individuals, I removed 16 breeding pen individuals and 9 free-range individuals based on their q-values. I then simulated hybridization between 37 breeding pen individuals and 49 free-range individuals; pairwise F_{ST} between stocks was 0.052 (Table 1). Average ancestry proportions for each parental and hybrid generation ranged from 0.173 to 0.840 (Fig. 1). The 90% confidence intervals revealed overlaps between F2-backcrosses and both F1s and the respective parental population. However, parental populations were differentiated from each other and from the F1 hybrids (Fig. 1).

Comparisons Between Breeding Pens, Enclosures, and Free-range Populations

USEPOPINFO Method

For my comparison using the MS-1 data, I assessed 14 pure breeding pen individuals, 29 pure free-range individuals, and 52 unknown enclosure individuals. Within the enclosure, I found that 11 individuals were assigned to the breeding pen, 5 to the F1, and 25 to the free-range population based on my 90%-confidence intervals (Fig. 2). Of the pure free-range population, 4 individuals fell outside of the 90%-confidence interval for free-range deer. All pure breeding pen individuals fell within the 90%-confidence interval for breeding pen deer.

For my comparison using the MS-2 data, I assessed 18 pure breeding pen individuals, 40 pure free-range individuals, and 51 unknown enclosure individuals. Within the enclosure, I found that 4 individuals were assigned to the breeding pen, 10 to the F1, and 35 to the free-range population based off of my 90%-confidence intervals (Fig. 2). Of the pure free-range population, 33 individuals fell outside of the 90%-confidence interval for free-range deer. All but 1 pure breeding pen individuals fell within the 90%-confidence interval for breeding pen deer.

For my comparison using the MS-3 data, I assessed 21 pure breeding pen individuals, 24 pure free-range individuals, and 22 unknown enclosure individuals. Within the enclosure, STRUCTURE assigned 0 individuals to the breeding pen, 1 to the F1, population and 21 to the free-range population based off of my 90%-confidence intervals (Figure 2). Of the pure breeding pen and free-range populations, all individuals fell within their respective 90%-confidence intervals.

LOCPRIOR Method

For all 3 comparisons, parental populations fell within their respective 90%-confidence intervals (Figure 3). Additionally, all enclosure individuals fell within the 90%-confidence interval for the free-range population except for 2 individuals from MS-2. The first of these MS-2 individuals

fell within the F1 90%-confidence interval with a q-value of 0.681 and the second fell within the breeding pen 90%-confidence interval with a q-value of 0.195.

DISCUSSION

Using average ancestry proportions and 90% confidence intervals, I successfully differentiated F1 populations from their respective breeding pen and free-range parental populations in all paired comparisons. However, all F2 populations overlapped 90% confidence intervals with their respective parental populations and the F1 hybrids. These findings are similar to the diminishing ability to differentiate beyond F1 hybrids in mule deer and blacktail deer (*Odocoileus hemionus*; Latch et al. 2011) and red wolves (*Canis rufus*) and coyotes (*C. latrans*; Bohling et al. 2013). Though I was able to differentiate parental breeding pen and free-range populations from each other and from their F1 hybrids, any further backcrossing would render differentiation impossible. With generational intervals as low as 2 years in white-tailed deer (Demarais et al. 2000), genetic detection of hybridization within white-tailed deer may only be effective out to five years depending on how intensive the release of non-native individuals have been.

In my empirical comparisons with enclosure populations using the "USEPOPINFO Method," I also identified the presence of known free-range individuals whose ancestry proportions fell outside the 90%-confidence interval for free-range populations for both MS-1 and MS-2 comparisons. STRUCTURE can assign admixture to individuals within populations known to be of pure descent (Vaha and Primmer 2006, Bohling et al. 2013), but I do not believe those free-range individuals show evidence of admixture resulting from introgression of breeding pen individuals. Rather, this may be due to both the geographic separation between the breeding pens and free-range populations, as well as, in the case of MS-1, MS-2, and MS-3, a 2 decades between sampling events. Additionally, the levels of differentiation between breeding pens and

free-range deer were lower than those found in similar studies using two different species (Latch et al. 2011, Bohling et al. 2013). Latch et al. (2006) discusses the difficulty STRUCTURE has assigning populations with low genetic differentiation and, in this case, there may not be enough of a distinction between deer populations to accurately assess hybridization. Finally, high levels of admixture in free-range populations of deer due to historic restocking efforts have been documented (DeYoung et al. 2003, Sumners et al. 2015, Chapter 1). This admixture may further complicate interpretation of my results.

Using the "LOCPRIOR Method", I was able to eliminate admixture within parental populations. Additionally, I greatly reduced the number of enclosure individuals that were assigned to generational subgroups. Therefore, this method performs more conservatively than the "USEPOPINFO Method." Despite the loss of assignment power, I was still able to identify two hybrid individuals in the MS-2 enclosure using this method. The "LOCPRIOR Method" may be best suited in cases where a conservative analysis is required to assess potentially non-native individuals, especially when high levels of admixture are present in the parental populations.

As introgression between breeding pen and free-range populations of white-tailed deer occurs, wildlife managers are interested in assessing the impacts of captive deer on native populations. In cases where managers release only a few individuals into a population of free-range deer, the resulting genetic impact may be diluted over time. My findings point to a rapid decline of detectability of breeding pen deer after 1 generation of hybridization. This means that if managers want to capture introgression events, they need to sample individuals within 2-5 years of release.

Further detection of hybrids may be possible with the advent of next-generation genetic sequencing such as single nucleotide polymorphisms (SNPs). Studies have found that a with a

moderate increase in the number of markers used, SNPs can outperform microsatellites in evaluating population structure (Liu et al. 2005, Smith et al. 2007, Morin et al. 2009). Additionally, Morin et al. (2009) discussed the inordinate effect that sample size has on increasing the statistical power of population differentiation. Therefore, it is likely that use of an appropriate number of SNPs as well as increasing the sample size of parental populations may have increased my ability to assay parental genetic variation and ultimately differentiate further generations of hybridization.

MANAGEMENT IMPLICATIONS

With the rise of the captive-cervid industry, state agencies are interested in better understanding the genetic consequences of mixing native and non-native populations of white-tailed deer. In particular, the ability to distinguish captive deer from free-range populations would be beneficial for tracking the illegal transfer of deer and identifying the origins of single individuals. However, the restocking histories of free-range deer and the variable management and breeding practices of the breeding pens make detecting non-native individuals difficult especially if populations are not genetically differentiated. Using 13-14 microsatellite DNA loci, I found that after 1 generation of hybridization between captive and free-range populations, my ability to distinguish F2-backcrosses faded. Further detection of hybrids may be feasible with the use of single nucleotide polymorphisms (SNPs) and an effort should be made to develop markers for white-tailed deer. However, even with these advanced technologies, each situation brings unique variables due to the restocking histories and management practices of specific deer populations. Care should be taken when interpreting results.

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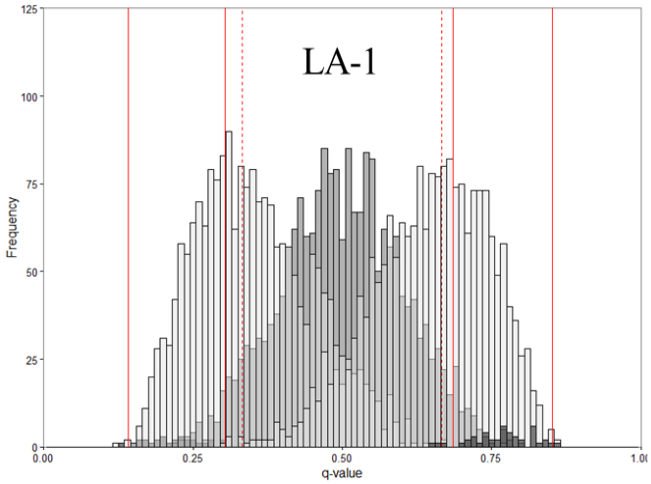
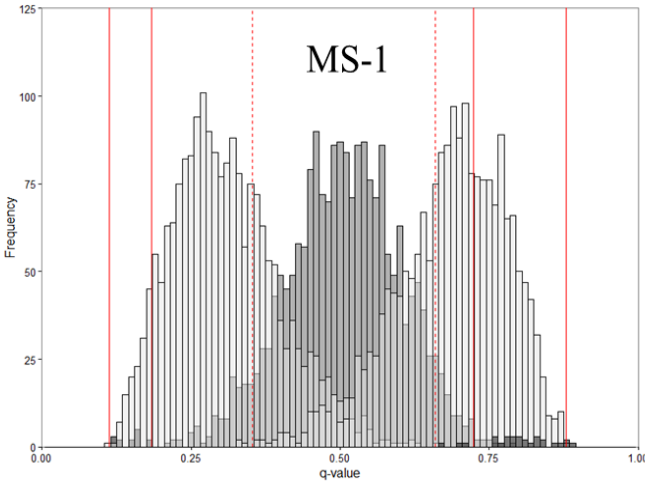
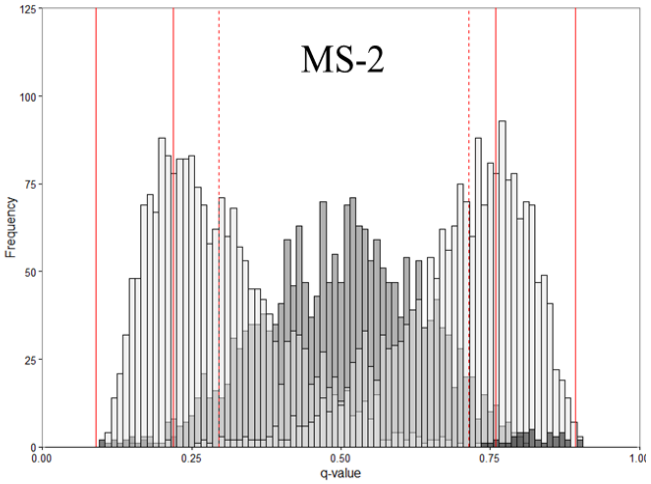
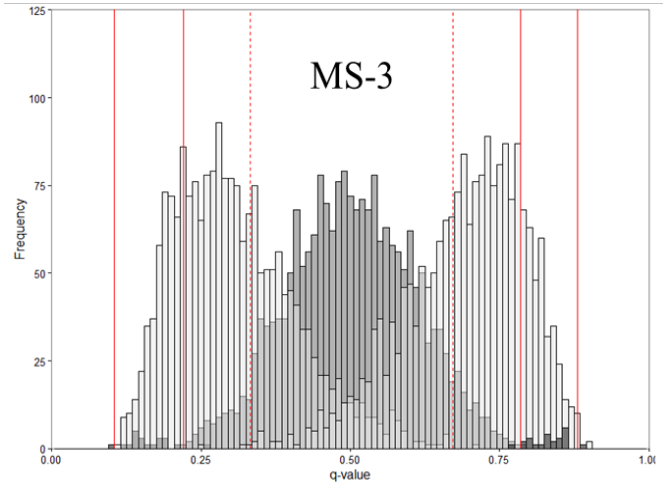
Table 1. Genetic differentiation (Nei's F_{ST}; Nei 1973) between paired populations of breeding pen and free-range white-tailed deer in southcentral U.S. Samples came from 1 breeding pen in Louisiana (LABP), 4 breeding pens in Mississippi (MS-1BP, MS-2BP, MS-3BP, MS-4BP), and 1 breeding pen in Alabama (ALBP). Pairwise free-range populations are denoted by "FR" in the upper horizontal heading.

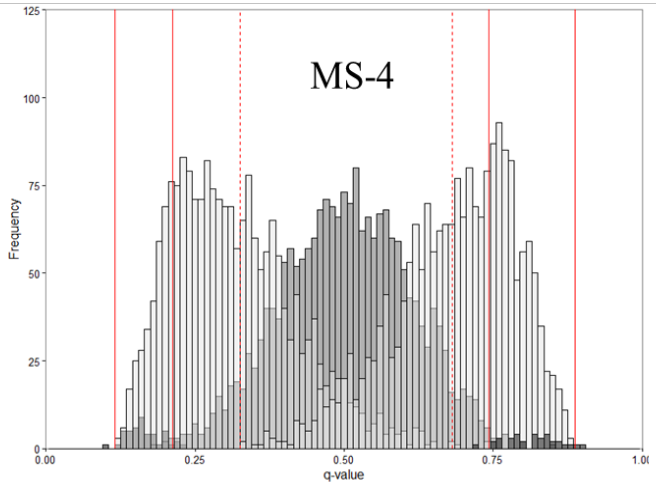
	LAFR	MS-1FR	MS-2FR	MS-3FR	MS-4FR	ALFR
LABP	0.045					
MS-1BP		0.050				
MS-2BP			0.036			
MS-3BP				0.068		
MS-4BP					0.060	
ALBP						0.052


LA-1
Frequency
q-value


MS-1
Frequency
q-value


MS-2
Frequency
q-value


MS-3
Frequency
q-value


MS-4
Frequency
q-value

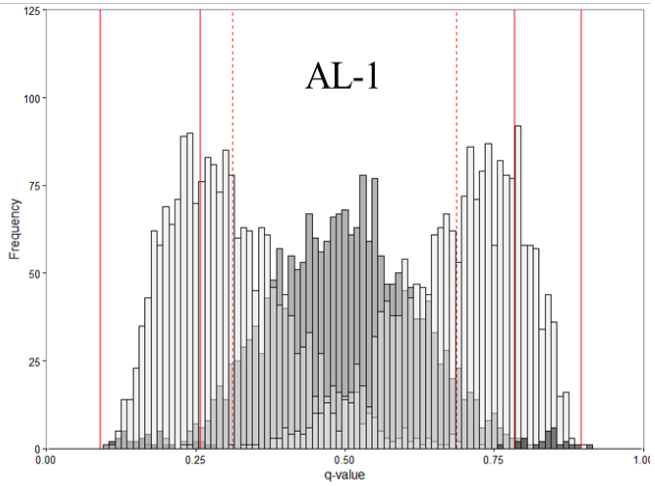

AL-1
Frequency
q-value

Figure 1. Distributions of ancestry coefficients (*q*-values) derived from STRUCTURE of 6 comparisons of breeding pens, free-range populations that have been subset to represent “pure” parental generations, and simulated F1 and F2 hybrids of white-tailed deer in southcentral U.S. Breeding pen and free-range populations are in black with breeding pens on the left and free-range populations on the right. Simulated F1 hybrids are in dark grey and F2 backcrosses are in light grey. The 90%-confidence intervals for parental populations are denoted by the solid red vertical lines and 90%-confidence intervals for F1 hybrids are denoted by the dashed red vertical lines. The 90%-confidence intervals for F2 populations are not shown because, in all cases, they overlapped with both parental and F1 populations.

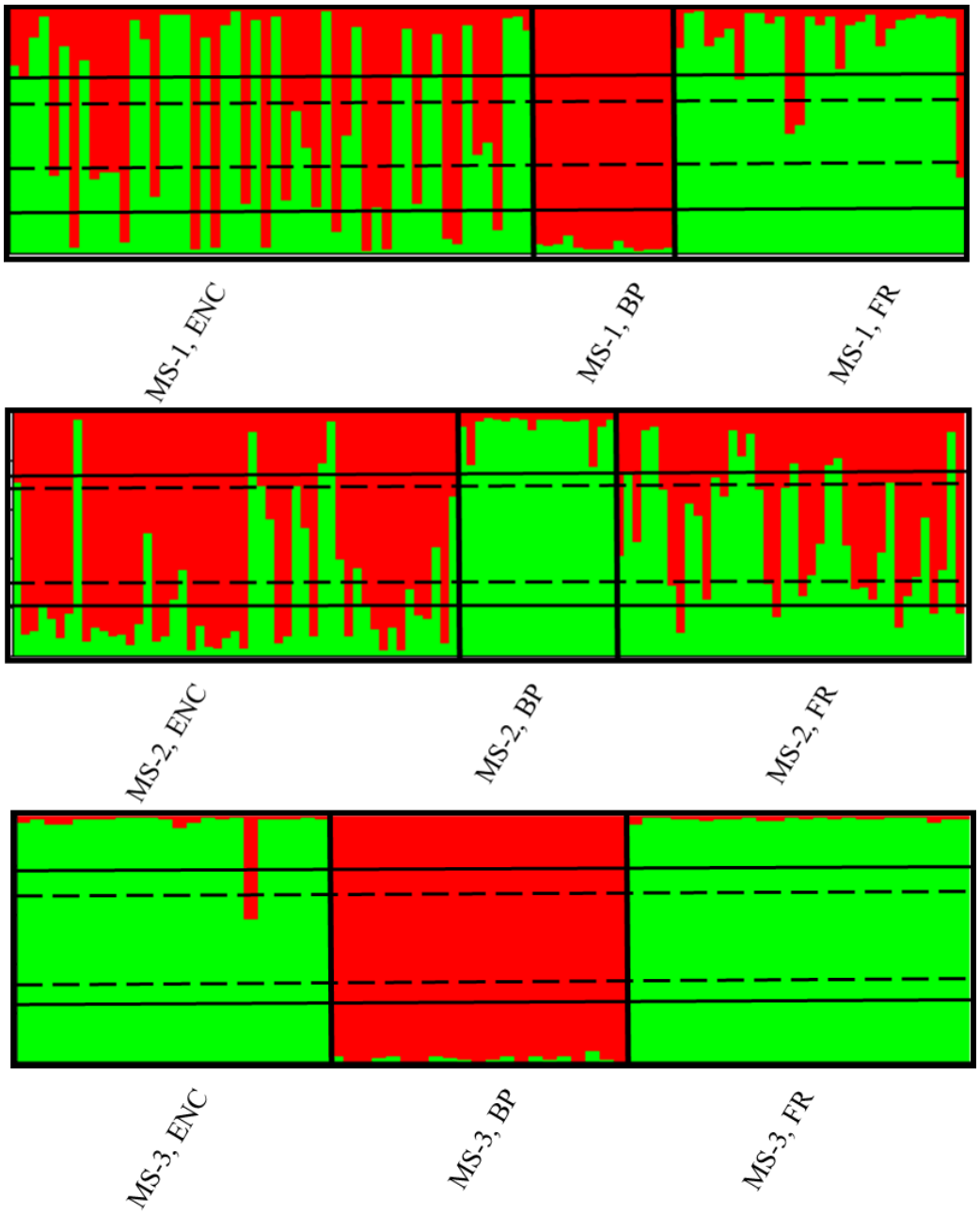


Figure 2. STRUCTURE output using the "USEPOPINFO Method" at K=2 for 3 comparisons of breeding pen (BP, high-fence enclosures (ENC), and adjacent free-range populations (FR) of white-tailed deer in southcentral U.S. The 90%-confidence intervals for breeding pen and free-range populations are denoted by the solid horizontal line and the 90%-confidence intervals for F1 hybrids are denoted by the dashed horizontal line.

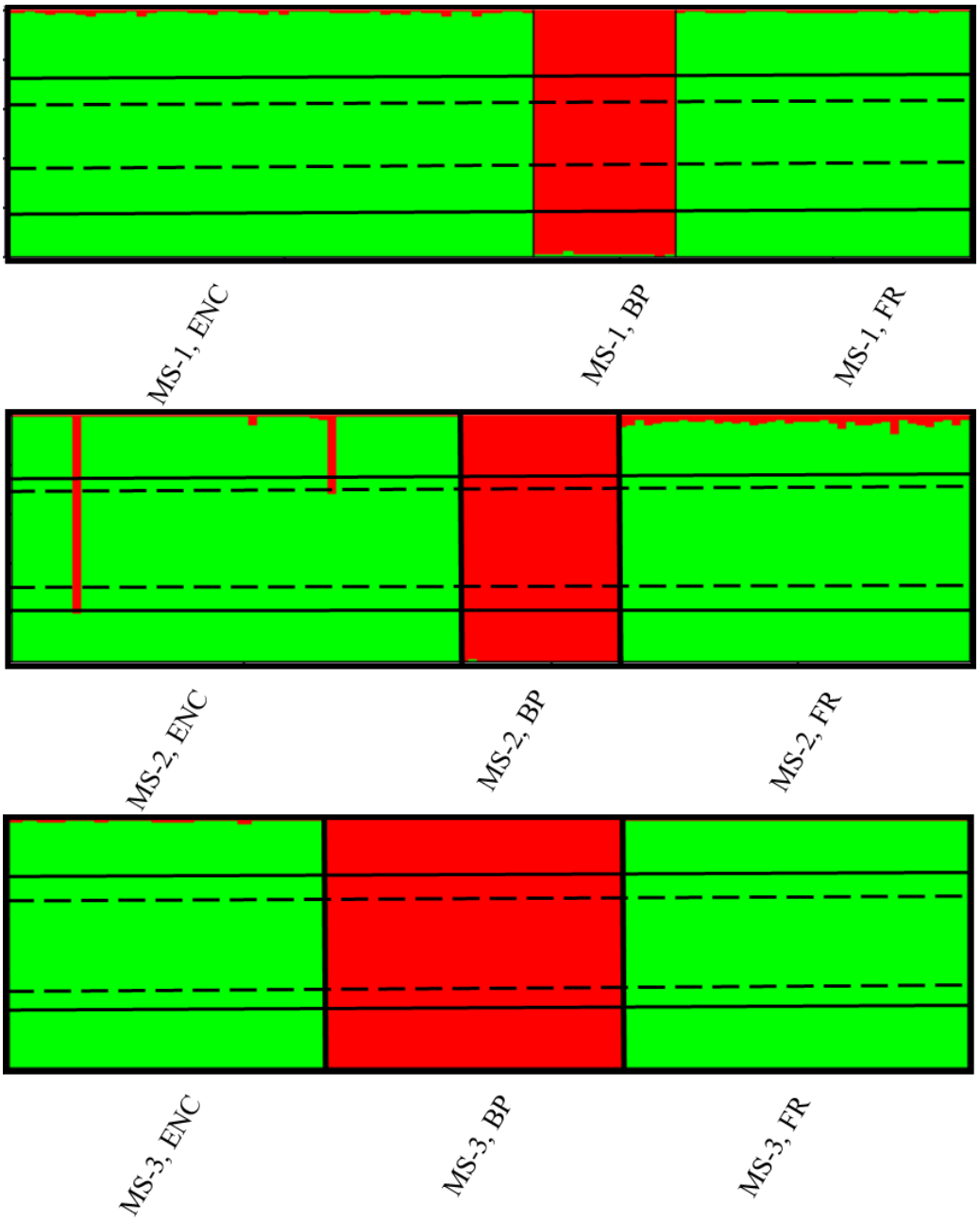


Figure 3. STRUCTURE output using the "LOCPRIOR Method" at K=2 for 3 comparisons of breeding pen (BP), high-fence enclosures (ENC), and adjacent free-range populations (FR) of white-tailed deer in southcentral U.S. The 90%-confidence intervals for breeding pen and free-range populations are denoted by the solid horizontal line and the 90%-confidence intervals for F1 hybrids are denoted by the dashed horizontal line.

CHAPTER 3

PROTOCOL TO DIFFERENTIATE NON-NATIVE WHITE-TAILED DEER IN FREE-RANGE POPULATIONS USING GENETIC METHODOLOGIES

INTRODUCTION

The rapid growth of the captive deer industry from 7,000 to about 10,000 breeding pen operations in the last ten years (Anderson et al. 2007, Adams et al. 2016) has raised concerns about potential impacts on free-ranging populations of white-tailed deer throughout the United States. The confinement and breeding of deer raises concerns, including the genetic consequences of inbreeding through extreme forms of husbandry and the increased risk of transmission of diseases (Geist 1985, 1988, Demarais et al. 2002).

Chronic wasting disease (CWD) is the main concern among the transmissible diseases. Chronic wasting disease is a transmissible spongiform encephalopathy (TSE) spread by abnormal prions through direct contact with an infected animal or prions deposited in the environment (Williams 2005, Gilch et al. 2011). Chronic wasting disease distribution has expanded from just 2 states prior to 2000 to its presence in 24 U.S. states (United States Geological Survey 2018). Detection in Mississippi in early 2018 reflects the furthest expansion into the southeastern US. Due to the nature of CWD transmission, captive facilities are uniquely prone to infection due to increased densities within enclosures and the introduction of individuals or reproductive products from regions where CWD is present.

The risk of CWD has led state agencies to minimize transport of live deer between captive facilities and mitigate interaction between captive and wild populations. Although 21 U.S. states banned the transportation of live, captive cervids across state boundaries (Sabalow 2014), regulation cannot account for illegal transport of live deer across state borders. For

example, owners of 2 captive cervid facilities were convicted of transporting live deer into Mississippi from Indiana and Pennsylvania (United States Department of Justice 2014). Deer held within breeding pens were later released into an enclosure, making it difficult to distinguish between wild and captive individuals. Clearly, there is need for effective testing protocols to identify captive stocks and their offspring from wild deer.

Genetic markers, such as DNA microsatellites, may provide enough resolution to discern the introduction of non-native deer into an enclosure (DeWoody et al. 1995, Anderson et al. 2002, DeYoung et al. 2003a, DeYoung and Honeycutt 2005). However, back-crosses may be difficult to distinguish from wild stocks. I was unable to accurately distinguish back-crosses (F2) from wild stocks (Chapter 2). The demographic history of deer in the southeastern US poses an additional challenge. Additionally, identifying genetic contributions from captive stocks is further complicated because historical restoration in the United States involved release of deer rom some of the same regions that provide breeding sources used in the captive-deer industry (Blackard 1971, McDonald and Miller 2004).

My objective was to formulate a protocol to differentiate captive from wild stocks in the southeastern U.S. This protocol can be used by wildlife managers to monitor and regulate the importation of genetic stock across state boundaries as well as investigate instances of illegal transportation of live deer. Additionally, this protocol may prove useful to organizations such as Boone and Crockett and Pope and Young, who require that animals be free-range in order to qualify for entry into record books. The ability to confirm a genetic source of deer would give such institutions a tool for verifying free-range status. Finally, the protocol may provide a way to document sources of disease by tracking the transportation of individuals from locations already infected with CWD.

I evaluated the efficacy of my methodologies to determine genetic origin of deer using 2 empirical case studies. The first case study was the first CWD-positive deer in Mississippi. The second case study was a road-killed doe found with ear-tags near a game-fenced enclosure in southern Mississippi. My goal was to determine whether the deer was of local origin or a product of a breeding pen. Both cases help to highlight both the strengths and weakness of my protocol and will help to inform future managers in how to conduct their own genetic comparisons.

STUDY DESIGN

To determine a population of origin for a single individual, I sampled native, free-range populations immediately surrounding where the unknown individual had been found. These free-range populations served as a baseline for genetic variation. I also sampled any captive facility that the unknown individual may have originated from. This allowed me to have representation of non-native genetic variation. Using both native and non-native populations allows the unknown individual to "match" its genetic variation to the most likely population of origin.

Case Studies

CWD-positive individual in Issaquena County, MS

A CWD-positive white-tailed deer was found on private property in Issaquena County, MS during the winter of 2018. Issaquena County is bordered on the western side by the Mississippi River and on the eastern side by Sharkey, Yazoo, and Warren Counties. East Carroll and Madison Parishes lie on the Louisiana side of the river, adjacent to Issaquena County. My native sample populations came from Sunflower WMA in Sharkey County, MS, about 30 kilometers away from where the CWD individual was found, and the Tensas National Wildlife Refuge in Madison Parish, approximately 50 kilometers away. I also included samples from the Noble Foundation, OK to provide geographic coverage as well as samples from a Louisiana breeding

pen approximately 80 kilometers away to compare the unknown deer against. This breeding pen was not suspected to be the origin of the CWD-positive deer but I included it as a representation of a captive facility, where number and origin of deer and management are unknown. Samples from Sunflower WMA came from archived data from DeYoung et al. (2003) and were collected from 1998-1999.

Tagged individual in southern Mississippi

The road-killed doe was found in southern Mississippi. My native sample populations came from Marion, Lamar, and Pearl River Counties in Mississippi and are all within 80 kilometers from where the doe was found. I also included 2 breeding pen populations from captive facilities in Pearl River and Lamar counties. My unknown individual was not suspected to have originated from either of these breeding pens, but they represent geographically-proximate, captive stocks. Samples from Pearl River County also came from archived data from DeYoung et al. (2003) and were collected from 1998-1999.

Sample Collection

Free-range populations

State agency employees or their agents collected tissue samples from free-ranging deer harvested during the 2015-16 and 2016-17 deer hunting season (archived samples from DeYoung et al. (2003a) were collected in 1998-1999). State biologists placed each tissue sample into a labeled bag and froze them. Upon receipt, I stored samples at Mississippi State University at -20ºC until DNA extraction. I then stored extracted samples at -80ºC.

Captive populations

I obtained both hair and tissue samples from breeding pen and enclosures. I stored hair samples in individual-labeled paper envelope. I then stored hair samples inside sealed ziploc bags

containing desiccant packets to protect them from moisture. I stored tissue samples in individually-labeled ziploc bags at -20ºC. Extracted DNA from both hair and tissue samples were stored at -80ºC.

DNA Extraction and Amplification

I isolated DNA from tissue samples using a commercial kit (DNeasy™ Tissue Kit, QIAGEN Genomics Inc., Hilden, Germany), following the manufacturer's protocol. I amplified 15 microsatellite DNA loci, including BL25, BM4208, BM6438, BM6506, BM848, Cervid1, ILSTS011, INRA011, and OarFCB193, D, K, N, O, P, and Q (Anderson et al. 2002, DeYoung et al. 2003b). I amplified loci in 4 multiplex reactions, as described by Anderson et al. (2002) and DeYoung et al. (2003a), and loaded the resulting products onto an automated genetic analyzer for separation and detection (3130*xl*, Applied Biosystems, Foster City, CA). I determined allele size calls for each locus using GeneMapper 4.0 (Applied Biosystems).

Data used from DeYoung et al. (2003) had been collected on a different sequencing platform than the one I used. To ensure that differences in migration of fragments did not affect allele size calls, I amplified and genotyped 71 individuals from DeYoung et al. (2003) as well as Sumners et al. (2015), which were chosen to be representative of the distribution of alleles detected in the studies. I used those genotypes to calibrate allele bins to ensure that microsatellite size-calls matched between data sets. I was unable to consistently assign allele calls for the Q locus; therefore I omitted that locus from further analyses.

Data Analysis

I calculated the average number of alleles, gene diversity (*H*; Nei 1987) and the inbreeding coefficient (F_{IS}, Weir and Cockerham 1984) for each site using FSTAT (Goudet 1995, 2002). I also tested for Hardy-Weinberg equilibrium and linkage disequilibrium within

populations and by locus using ARLEQUIN 3.1 (Excoffier et al. 2005). I corrected for multiple comparisons using a Bonferroni procedure. Population genetic theory articulates that recent admixture between different populations can inflate F_{IS} and linkage disequilibrium and, therefore, reveal introgression of stock sources (Wahlund 1928).

To assess population genetic structure, I computed pairwise F_{ST} among all sites using ARLEQUIN 3.1 (Excoffier et al. 2005), with significant differentiation between populations ($p \leq 0.05$) assessed based on 100 permutations of individuals between populations. I also tested differentiation among populations using Nei's genetic distance (D_A; 1983) with the R-based software package, ADEGENET (Jombart et al. 2010). Nei's D_A assumes no underlying evolutionary model, unlike F_{ST}, which may not perform well in the presence of admixture (Nei and Kumar 2000). It is, therefore, suited for analysis of populations wherein lineage mixing has occurred.

I used the Bayesian clustering algorithm implemented in STRUCTURE 2.3 (Pritchard et al. 2000, Pritchard et al. 2010) for both evaluations of unknown individuals with their respective comparison populations. I first ran exploratory analysis, without including the unknown individual in question, using the admixture, correlated allele frequency model to assess baseline genetic structure of my populations. I ran this analysis assuming 1-10 genetic clusters (K), with an initial burn-in of 50,000 Markov chain Monte Carlo (MCMC) repetitions, followed by 150,000 MCMC repetitions for data collection. I performed 5 iterations at each K.

I then ran an analysis including the unknown individual in question, using the admixture model and assuming correlated allele frequencies, I used the LOCPRIOR designation for the comparison populations, where sampling location acts as a weak prior to inform clustering (Hubisz et al. 2009). Using this methodology, I was able to model the CWD-positive individual

and the road-killed doe as unknowns so that their assignment would be informed by the allele frequencies of the comparison populations. I ran each comparison at genetic clusters (K) from 1–10, with an initial burn-in of 50,000 Markov chain Monte Carlo (MCMC) repetitions, followed by 150,000 MCMC repetitions for data collection. I repeated the runs for each K 5 times. I determined the most likely number of clusters based on the change in the likelihood function between each successive cluster (ΔK, Evanno et al. 2005) using the program STRUCTURE HARVESTER (Earl and vonHoldt 2012). I performed an additional series of runs at the most likely K, K-1 and K+1, to ensure model convergence. The additional runs consisted of a 100,000 MCMC burn-in followed by 200,000 MCMC repetitions of data collection, with 10 iterations at K, K-1, and K+1.

I also conducted an analysis of principal components using the Discriminant Analysis of Principal Components (DAPC) algorithm in ADEGENET (Jombart et al. 2008) implemented through Program R (R Core Team 2015). Multivariate analyses such as DAPC does not use population genetic theory and serves as a complement to Bayesian clustering methodologies. The number of groups in my DAPC were matched with the best-fit K from my STRUCTURE analysis so as to compare the structure solutions that both methods arrived at.

RESULTS

I analyzed 9 populations ranging in size from 19-33 individuals (Table 1). Average number of alleles ranged from 6.5-9.3, with the lowest in the MS-1 breeding pen and the highest in the Tensas National Wildlife Refuge, LA. Inbreeding coefficients (F_{IS}) ranged from -0.06-0.12 with the MS-1 breeding pen be the lowest and the highest being the MS-2 breeding pen. Average gene diversity (H) ranged from 0.70-0.76, with the lowest in the MS-1 breeding pen and the highest in Lamar County, MS. The MS-2 breeding pen exhibited the lowest number of loci in Hardy-

Weinberg equilibrium (11), while the Noble Foundation, OK, Tensas NWR, LA, Sunflower WMA, MS, Lamar County, MS, and the LA-1 breeding pen had all loci in Hardy-Weinberg equilibrium (14). Finally, the number of pairwise loci in linkage disequilibrium ranged from 0-5 where Tensas NWR, LA and Pearl River, MS had the lowest and the LA-1 breeding pen had the highest.

I quantified genetic differentiation using Nei's D_A, which ranged from 0.149-0.317. The lowest differentiation was between Marion County, MS and Pearl River, MS and the highest was between Sunflower WMA, MS and the MS-1 breeding pen. Measures of significant F_{ST} ranged from 0.011-0.075. The lowest differentiation was between Noble Foundation, OK and the MS-2 breeding pen and between Marion County, MS and the MS-1 breeding pen. The highest differentiation was between Noble Foundation, OK and Sunflower WMA, MS.

Analysis of the CWD-Positive Individual in Issaquena County, MS

In my preliminary analysis with STRUCTURE using the simple admixture, correlated allele frequency model, I found a best fit for ΔK at K=4 (Fig. 1). This population structure shows general differentiation between all 4 populations; Sunflower WMA, MS, Tensas National Wildlife Refuge, LA, Noble Foundation, OK, and the Louisiana breeding pen (Fig. 2). There was some admixture present in each population.

In my LOCPRIOR model analysis with the CWD-positive deer, I also found a best fit for ΔK at K=2 (Fig. 3). However, there was also an increase in ΔK at K=4 that appeared to be biologically feasible and so I continued my analysis at 4 clusters. At K=4, there was high differentiation between each of the comparison populations. The CWD-positive deer was assigned 80.1% with the Sunflower WMA, MS population and 14.1% with the Tensas National

Wildlife Refuge, LA population (Fig. 4). It was assigned to the Noble Foundation, OK population and the Louisiana breeding pen under 5%, respectively.

My DAPC analysis at 4 clusters corroborated the Bayesian assignments, as the CWD-positive individual clustered into Group 3, comprised of 90.3% Sunflower WMA, MS and Tensas National Wildlife Refuge, LA (Fig. 5). Group 3 overlapped with Group 4, which was comprised of 83.3% Sunflower WMA, MS and Tensas National Wildlife Refuge, LA. Group 3 and Group 4 were differentiated from Group 1 (81.8% Noble Foundation, OK and 18.2% Louisiana breeding pen), as well as from Group 2 (88.5% Louisiana breeding pen). Both Group 1 and Group 2 were also fully differentiated with no overlap.

Analysis of the Unknown Tagged Doe in Southern Mississippi

For my preliminary analysis with STRUCTURE using the simple admixture, correlated allele frequency model, I found a best fit for ΔK at K=4 (Fig. 1). Genetic structure at 4 clusters was admixed across all populations (Fig. 2). However, free-range populations, Marion County, MS, Lamar County, MS, and Pearl River County, MS, grouped together, while the 2 Mississippi breeding pens were different from both the free-range populations and each other.

My LOCPRIOR analysis including the unknown tagged doe showed a best fit for K=3 (Fig. 3). All free-range populations grouped together, while the 2 Mississippi breeding pens grouped separately from each other and the free-range deer. The unknown individual showed an average assignment of 37.9% with the free-range populations and 58.0% with one of the 2 breeding pens (Fig. 4).

My DAPC analysis at 3 clusters corroborated the Bayesian assignments, as the tagged doe clustered into Group 2, comprised of 50.0% free-range deer and 47.5% breeding pen deer

(Fig. 5). Group 1 was comprised of 92.0% breeding pen deer while Group 3 was comprised of 97.9% free-range deer. All 3 groups were clearly separated and showed no overlap.

DISCUSSION

I was able to assign individual unknown white-tailed deer to native and non-native origins using a combination of Bayesian assignment and multivariate methodologies. I also showed how breeding pen populations were distinct when compared to geographically proximate, free-range populations. This indicates that, in some cases, single individuals can be determined to be non-native if comparison populations are sufficiently differentiated and, more generally, that breeding pen populations can be genetically differentiated from free-range populations. However, interpretation of these results can be difficult due to factors including genetic admixture, low differentiation between captive and free-range populations, and the use of surrogate breeding pens for comparisons.

I found some evidence of admixture across all populations in my preliminary analyses, even within breeding pens. Admixture in free-range white-tailed deer populations in the southeast U.S. was attributed to lingering effects of restocking of non-native deer during the mid-1900s (DeYoung et al. 2003a, Sumners et al. 2015, Chapter 1). Additionally, some breeding pens will use native deer as stock for their animal husbandry efforts (William McKinley, MDWFP, *personal communication*). This can lead to genetic variation that is mixed between non-native and native lineages which complicates the analysis and affects the ability to unambiguously assign individuals to a population of origin (Chapter 2). Both these factors, the restocking history of white-tailed deer and the genetic manipulation of captive deer, can result in populations that share ancestry and are not highly differentiated from each other. Therefore, assigning individuals

to either a free-range or breeding pen population may be difficult if both comparison populations share ancestry.

An example of the ambiguity that can arise from comparing admixed, minimally differentiated populations can be seen in the assignment of the tagged doe that was found in southern Mississippi. The assignment of the tagged doe to both free-ranging and breeding pen deer by STRUCTURE and DAPC (Fig.4, Fig. 5) can be interpreted as the doe having a proportion of ancestry coming from a breeding pen population. However, considering that the MS-2 breeding pen exhibited admixture with the surrounding free-range populations in my preliminary analysis, genetic variation within that unknown doe may only be linked to free-range genetic variation in the MS-2 population. It, therefore, becomes difficult to tease out distinctions when all comparison populations show evidence of shared ancestry.

I also did not find high differentiation between populations and, specifically, breeding pens were not substantially differentiated from nearby free-range populations. Low differentiation has been shown to complicate interpretation of Bayesian clustering assignments (Latch et al. 2006) and, therefore care must be taking interpreting my STRUCTURE results. However, corroboration of my results by the multivariate, DAPC approach lends support to the overall trends in my results.

Finally, in both case studies, my inability to procure samples from breeding pens suspected of being source populations for the unknown individuals required that I used a surrogate breeding pen population as a stand-in for genetically manipulated individuals. This is a limitation to this kind of assignment methodology due to that chance of inaccurately assigning individuals to comparison populations when they share no ancestry (Cornuet et al. 1999, Manel et al. 2002). In the case of the tagged doe in southern Mississippi, I have no reason to believe that

the ancestry proportion assigned to the MS-2 breeding pen population actually represents descent (William McKinley, MDWFP, *personal communication*). Therefore, interpretation of both my data, and data collected under similar circumstances must be conducted with care. I recommend that managers attempting to assign unknown individuals to a population should sample suspected populations so as to provide a more accurate assessment.

Genetic technologies provide wildlife biologists with a suite of tools to enhance their ability to manage native populations (DeYoung and Honeycutt 2005). Through lowered costs and increased access to laboratories that can perform genetic analysis, molecular-based technologies that can answer specific management objectives will become increasingly useful to state and federal agencies. However, biologists need clear, replicable methods in order to use these new tools. My goal for this chapter was to showcase a methodology that managers could use to genetically distinguish non-native white-tailed deer in free-range populations. While the methods I used were relatively straightforward, interpreting results can be difficult.

MANAGEMENT IMPLICATIONS

As the captive-cervid industry has rapidly grown in the last few decades (Anderson et al. 2007, Adams et al. 2016), worry has grown over the potential for escaped, genetically-manipulated individuals being found in wild populations. Additionally, state agencies have begun to ban the shipment of live animals across state borders to combat the spread of diseases such as chronic wasting disease (Sabalow 2014). State biologists are interested in determining if illegal deer have been released. The methodologies laid out in this chapter can be useful in comparing individuals of concern to native populations. However, the restocking and animal husbandry histories of white-tailed deer, the low differentiation between populations, and the difficulty of procuring breeding pen samples can all confound interpretation of results. Managers need to develop robust

sampling strategies that provide a meaningful genetic baseline for comparison and then proceed with caution when analyzing their data. Finally, due to the ever changing nature of the captive-cervid industry and the variability found in free-range populations, these methodologies should be used on a case-by-case basis.

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Table 1. Summary statistics of genetic diversity including sample size (*N*), average inbreeding coefficient (F_{IS}) with standard deviation, average gene diversity (*H*) with standard deviation, and significant pairwise linkage disequilibrium (*LD*) for captive and free-range populations of white-tailed deer across southcentral U.S analyzed with 14 microsatellite DNA loci. Populations were sampled from 2015-2017 except for Noble Foundation, OK, Sunflower WMA, MS, and Pearl River, MS which were sampled in 1998-1999.

Population	*N*	A (SD)	F_{IS} (SD)	*H* (SD)	HWE	LD
Noble Foundation, OK	30	7.8 (2.8)	0.07 (0.16)	0.75 (0.14)	14	2
Tensas NWR, LA	30	9.3 (3.5)	0.02 (0.13)	0.74 (0.19)	14	0
Sunflower WMA, MS	20	7.7 (3.2)	0.04 (0.14)	0.74 (0.20)	14	2
Marion County, MS	23	8.2 (3.2)	0.06 (0.14)	0.74 (0.23)	12	1
Lamar County, MS	19	7.9 (2.9)	0.05 (0.12)	0.76 (0.22)	14	4
Pearl River, MS	29	8.9 (2.8)	0.09 (0.13)	0.75 (0.18)	13	0
LA-1 Breeding Pen	33	7.5 (2.7)	0.02 (0.10)	0.75 (0.15)	14	5
MS-1 Breeding Pen	19	6.5 (3.0)	-0.06 (0.11)	0.70 (0.17)	13*	1
MS-2 Breeding Pen	23	7.8 (3.8)	0.12 (0.16)	0.71 (0.27)	11*	1

*Monomorphic for ILSTS locus

Table 2. Pairwise genetic differentiation using Nei's D_A (upper diagonal) and F_{ST} (lower diagonal) between white-tailed deer populations from free-range and breeding pen populations in southern U.S. based on 14 microsatellite DNA loci. Pairwise F_{ST} relationships that were significantly different from 0 ($p \leq 0.05$) are designated by an asterisk in the lower diagonal. Populations were sampled from 2015-2017 except for Noble Foundation, OK, Sunflower WMA, MS, and Pearl River, MS which were sampled in 1998-1999.

	Noble Foundation, OK	Tensas NWR, LA	Sunflower WMA, MS	Marion County, MS	Lamar County, MS	Pearl River, MS	LA-1 Breeding Pen	MS-1 Breeding Pen	MS-2 Breeding Pen
Noble Foundation, OK	----------	0.194	0.258	0.208	0.241	0.198	0.193	0.249	0.193
Tensas NWR, LA	0.052*	----------	0.180	0.193	0.164	0.203	0.198	0.251	0.189
Sunflower WMA, MS	0.075*	0.041*	----------	0.248	0.236	0.226	0.256	0.317	0.213
Marion County, MS	0.030*	0.018*	0.036*	----------	0.158	0.149	0.199	0.277	0.253
Lamar County, MS	0.031*	0.012*	0.026*	0.014*	----------	0.161	0.225	0.266	0.244
Pearl River, MS	0.032*	0.032*	0.044*	-0.010	-0.014	----------	0.220	0.290	0.245
LA-1 Breeding Pen	0.042*	0.054*	0.062*	0.032*	0.014*	0.047*	----------	0.179	0.152
MS-1 Breeding Pen	0.018*	0.025*	0.050*	0.011*	-0.005	0.053*	0.045*	----------	0.187
MS-2 Breeding Pen	0.011*	0.024*	0.022*	0.025*	-0.011	0.038*	0.023*	0.049*	0

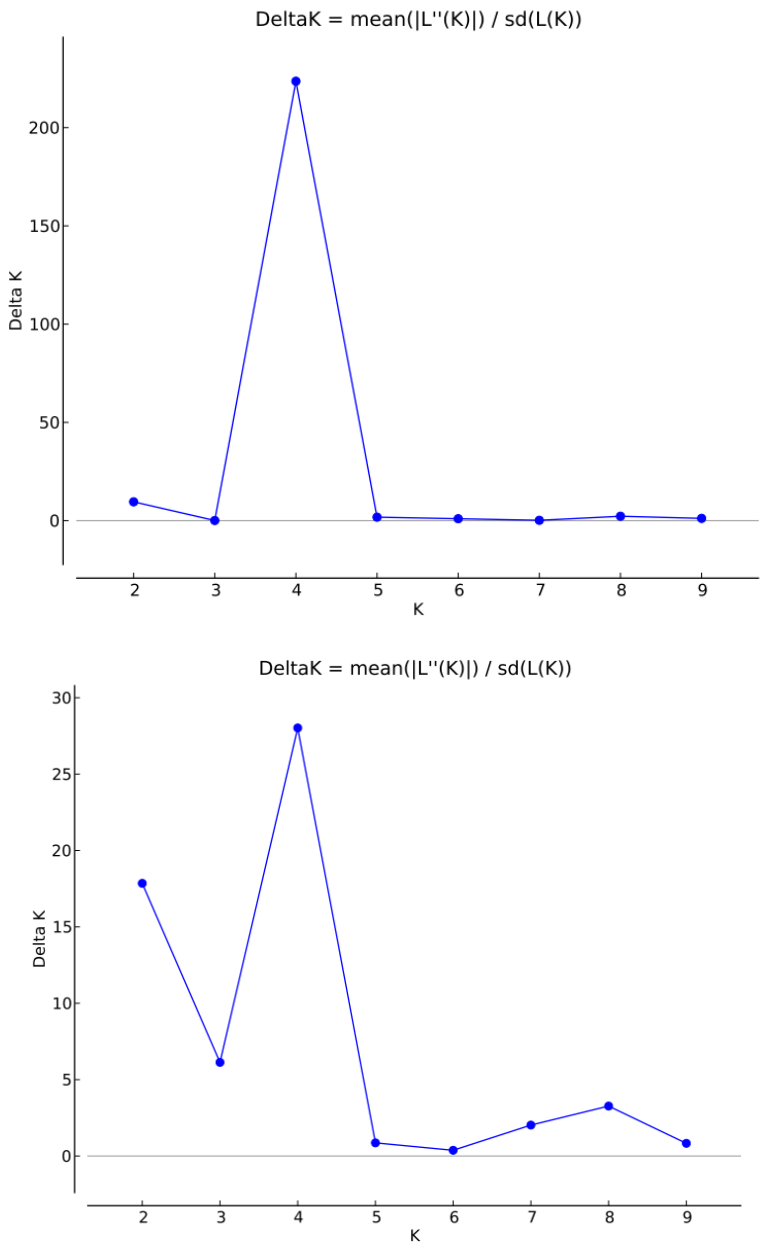

Figure 1. Evanno et al. 2005's comparison of the second order rate of change (ΔK) of the log probability of data obtained from STRUCTURE analysis using the admixture and correlated allele frequency models. Peaks in ΔK can be used as an ad hoc determination of the most likely number of clusters (K). Analysis compared the the white-tailed deer populations surrounding the CWD positive deer in Issaquena County, MS (top; K=4) and the populations surrounding the tagged roadkill doe in southern Mississippi (bottom; K=4).

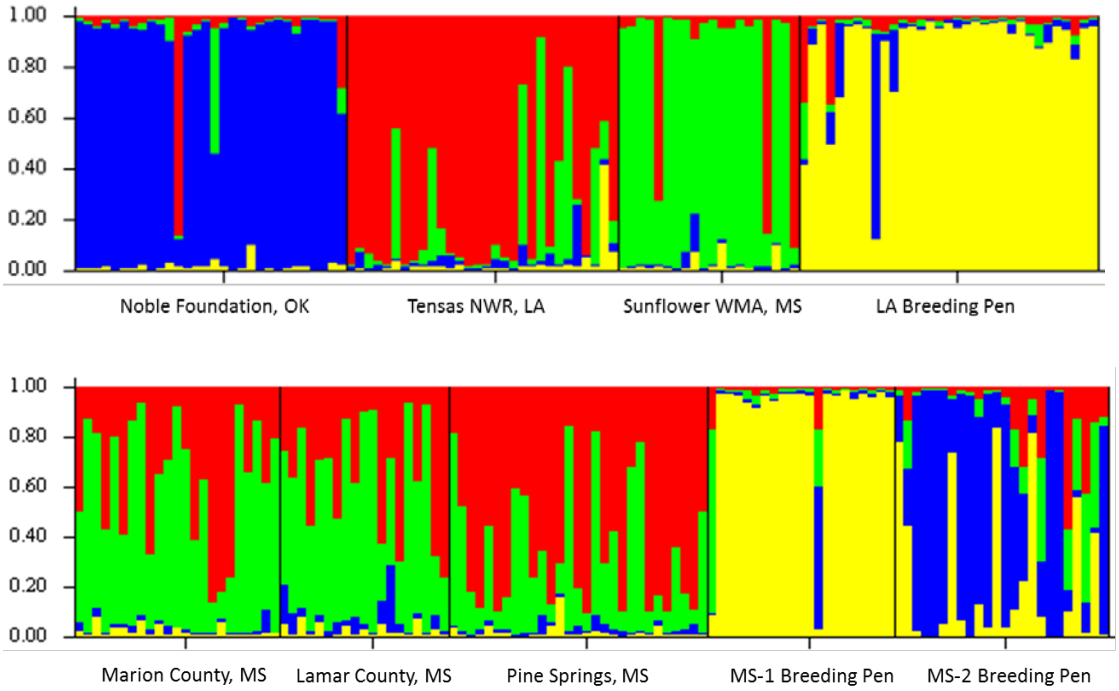

Figure 2. Preliminary STRUCTURE analysis using the admixture and correlated allele frequency models for the white-tailed deer populations surrounding the CWD positive deer in Issaquena County, MS (top) and the populations surrounding the tagged roadkill doe in southern Mississippi (bottom). Using 14 microsatellite DNA loci, this analysis assigns ancestry proportions for individuals represented by the vertical bars. Populations were sampled from 2015-2017 except for Noble Foundation, OK, Sunflower WMA, MS, and Pearl River, MS which were sampled in 1998-1999.

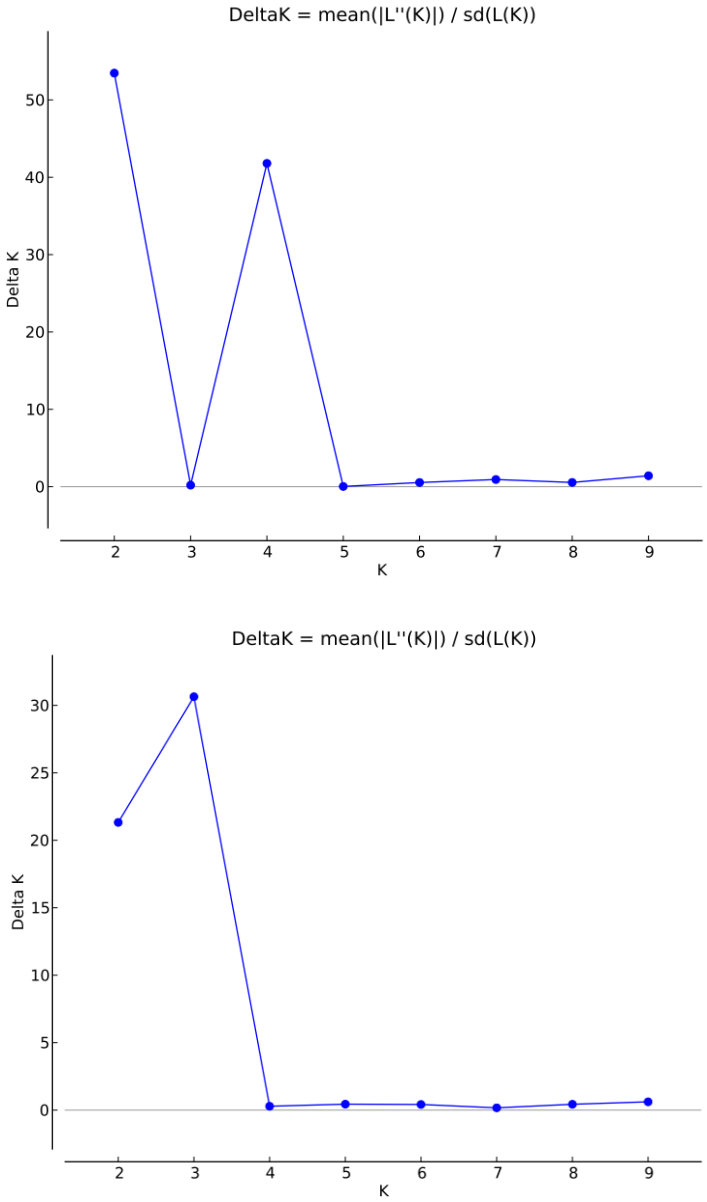

Figure 3. Evanno et al. 2005's comparison of the second order rate of change (ΔK) of the log probability of data obtained from STRUCTURE analysis using the admixture, correlated allele frequency, and LOCPRIOR models. Peaks in ΔK can be used as an ad hoc determination of the most likely number of clusters (K). Analysis compared the CWD positive white-tailed deer in Issaquena County, MS and surrounding populations (top; K=4) and the tagged roadkill doe in southern Mississippi and surrounding populations (bottom; K=3) using 14 microsatellite DNA loci.

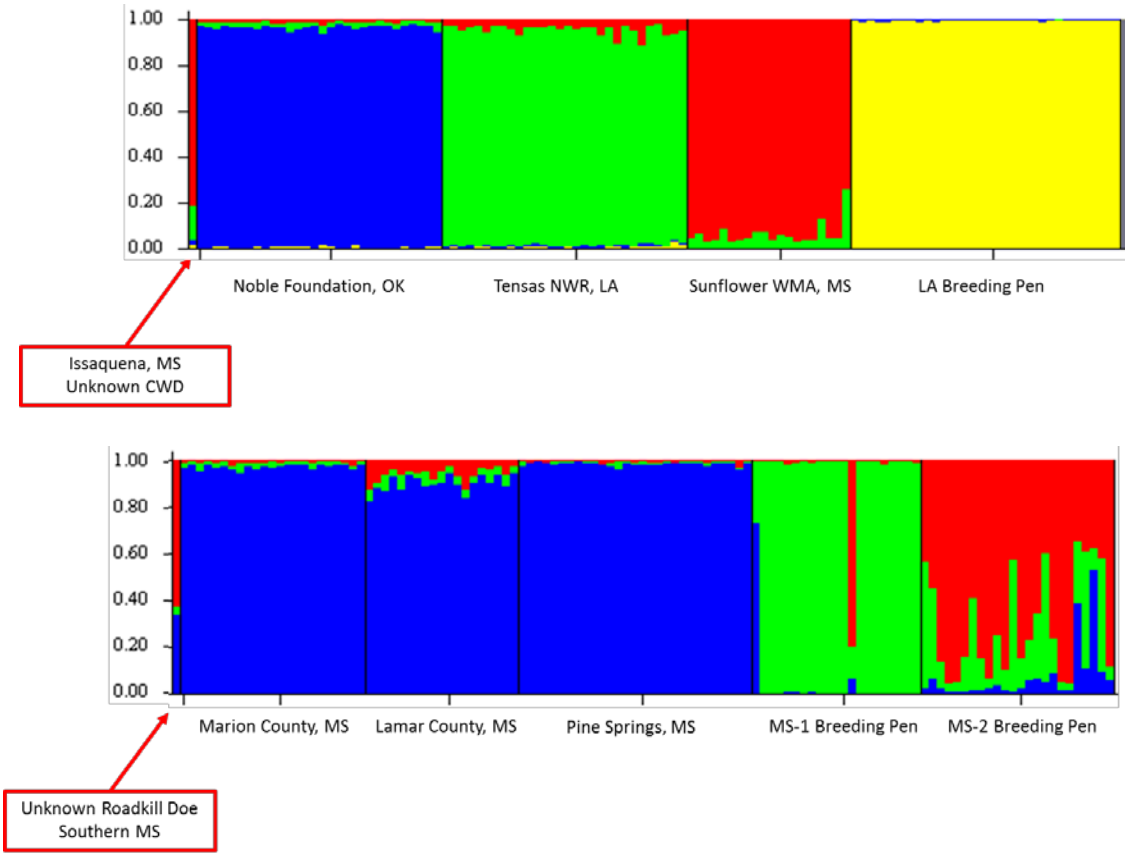

Figure 4. STRUCTURE analysis for comparisons between the CWD positive white-tailed deer in Issaquena County, MS and surrounding populations at K=4 (top) and the tagged roadkill doe in southern Mississippi and surrounding populations at K=3 (bottom). Both comparisons used the LOCPRIOR model by designating surrounding populations as a prior to inform the assignment of the unknown individual. Using 14 microsatellite DNA loci, this analysis assigns ancestry proportions for individuals represented by the vertical bars. Populations were sampled from 2015-2017 except for Noble Foundation, OK, Sunflower WMA, MS, and Pearl River, MS which were sampled in 1998-1999.

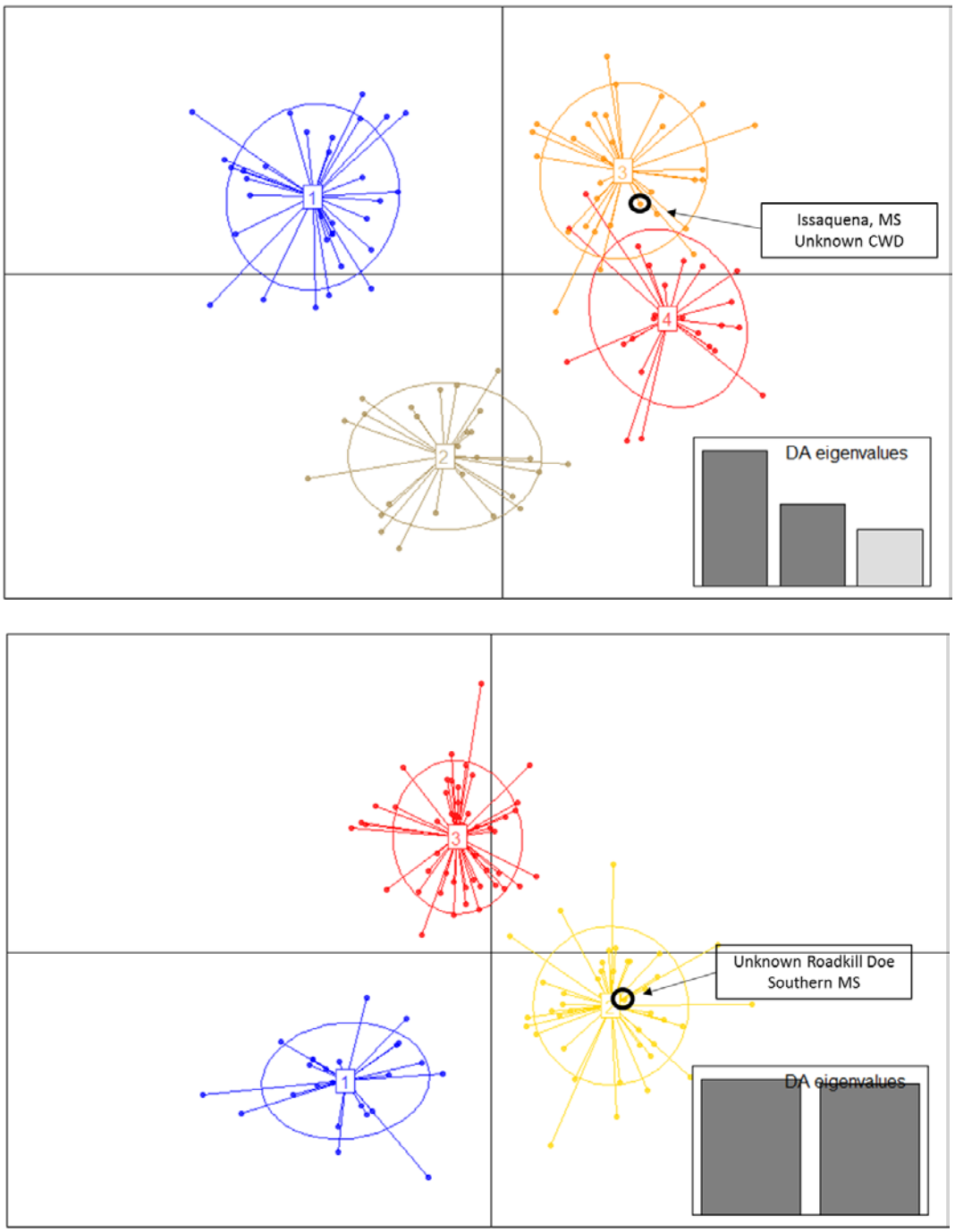

Figure 5. Discriminate Analysis of Principal Components (DAPC) from the ADEGENET software showing clustering of the CWD positive white-tailed deer in Issaquena County, MS and surrounding populations at 4 groups (top) and the tagged roadkill doe in southern Mississippi and surrounding populations at 3 groups (bottom). This analysis used 14 microsatellite DNA loci.