SIKA DEER INTRODUCTION, POPULATION GENETICS, AND THEIR COMPETITION WITH WHITE-TAILED DEER

by

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A dissertation submitted to the Faculty of the University of Delaware in partial
fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology
and Wildlife Ecology

Summer 2015

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It is God who arms me with strength and keeps my way secure. He makes my feet like the feet of a deer; he causes me to stand on the heights. He trains my hands for battle; my arms can bend a bow of bronze. You make your saving help my shield, and your right hand sustains me; your help has made me great. You provide a broad path for my feet, so that my ankles do not give way.

~Psalms 18:32 – 36.

To: Theodore Chester Kalb

Without a word you have inspired me to be the best person I am able.

ACKNOWLEDGMENTS

I would like to thank first off and most importantly, my wife, who has aided in this project from conception to the final paragraph. The work was not always fun, but together we always enjoyed the moments. During this time we managed to fall in love get married and start a family, so in a way this dissertation is a testament to what we can do together. Thank you Melissa, you have all my love, always.

I would also like to thank my friends and my family for their unwavering love and support. These people instilled the love of learning about nature and conservation in me. Thank you for everything you have done, imparted, and encouraged, too numerous to mention here. I hope that they are proud to see how my love of the natural world, and I have bloomed.

Thanks to all my fellow graduate students who have been immeasurably supportive in every way. I also thank the undergraduate students who were excited to work on this project and were a great aid in the laboratory during several semesters (T. Bonner, H. Kline, L. Newton, K. Lewis, L. Caprini, and C. Blakely). This project would not have been possible without their enthusiasm and help.

There are many private landowners and managers who allowed access to their properties at many different times of the year, I would like to acknowledge them here; M. Aiello, M. Achenbach, H. Hill, P. Hobbs (including A. Loid), P. Jones (including A. Jolicoeur and K. Compton), C. Kahl, P. Ransome (including M. Willey), J. Ridgley, R Riggins, T. Strong, M. Strong, and D. Twigg. I would also like to thank Dr. H. Tamate from Yamagata University and associates for the kind donation of sika deer DNA samples from Yakushima Island. These samples were critical in supporting the hypothesis of this work. I thank Dr. B. Vasilas from the University of Delaware, for the time investment to provide expertise on field site hydrology, soils, and plant communities.

Thank you to the University of Delaware and the Department of Entomology and Wildlife Ecology for the experience and financial support. I would finally like to thank my committee members who have offered guidance and motivation throughout this project. At times you were voices of support and confidence, and when necessary you were harsh critics. I thank you for reining me in when I was running crazy, and pushing me harder when I was lagging. Each of you contributed to this in ways that make the final product as much yours as mine. I would also like to pay especial acknowledgement to Dr. Shriver and Dr. Bowman who were gracious enough to serve on my committee for two degrees which at times, I am sure, was not always an easy task. You have all changed my life for the better.

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ABSTRACT

The ecological problems caused by invasive species are not new. Over time these species have been labeled in several ways, however they are labeled; non-native, exotic, non-indigenous, or alien, species that establish populations outside of their native range cause changes in the balance of the natural ecosystem. The negative effects of an introduction can go unnoticed for generations, and once we realize the problem, it is often too late to control. In these situations, we as stewards to the natural environment must manage the new species. Proper management requires not only a knowledge of the species biology, ecology etc., but also a working understanding of how these species affect the native ecosystem.

Ungulates are the most commonly introduced animal species in the world. Whether for controlled/contained hunting or the intentional introduction into wild populations, cervids (deer) are a popular non-native species worldwide (Dolman and Wäber 2008). While currently in several states (captive and free ranging), the first introduction of sika deer, was actually in Maryland. Sika deer were first introduced to the United States as an additional game species when our native white-tailed deer population numbers were at an all-time low. Their introduction was not noticed or was overlooked for 4 decades, until the 1950's when they began to rapidly increase in number. The belief that sika deer could coexist with white-tailed deer was accepted, and applauded as some considered sika deer a replacement and/or additional game species for areas that no longer retained white-tailed deer. However, as sika deer

continue to expand their range and increasing in number, this notion needs to be more thoroughly addressed.

Until now the history of sika deer in Maryland has started at James Island, the point of original release into the wild. Here I investigate the complete history of how sika deer came to Maryland from their native Asia, and their spread to both shores of the Delmarva Peninsula. I trace the lineage of sika deer in Maryland back to stocks in the UK from a population in Japan. I also highlight the key players in multiple countries that have led to the establishment of sika deer on both the eastern and western sides of the Delmarva Peninsula.

With an understanding of their stocking source I investigate the genetic fitness of these sika deer and implications that result from the founder events and natural changes in population. I compared the molecular variation of sika deer on the Delmarva Peninsula to those of the source population on Yakushima Island, Japan and to captive held sika deer in Delaware using 10 microsatellite markers. Through a series of founder events and population bottlenecks, condensed into a few generations sika deer in Maryland are depauperate in neutral allelic diversity. I observed 14 alleles (including two unique) in the Dorchester population, and 11 alleles in the Assateague population (none unique). The paucity of variation that does exist alludes to a history of hybridization or genetic admixture as well as a period of invasive lag once the deer were release in Maryland. The genetic diversity I observed support that limited variation that has existed in neutral alleles of sika deer since glacial periods of the Precambrian. The allelic richness and levels of heterozygosity are similar to those observed in other severely bottlenecked populations or populations founded from few individuals. I used the aforementioned pre-introduction information to create an approximate Bayesian computation (ABC) of the demographic history of Maryland's sika deer, including estimations of current and historic effective breeding population sizes. ABC supported a single introduction to the Delmarva by way of a sika deer introduced to the UK from Japan. Multiple founding of a large population from few individuals in a short period of time has resulted in changes in genetic diversity, and rates of change within population growth. My results from ABC modeling support a rapid population expansion in the UK, then a slower growth in numbers, and longer generational periods once sika deer were brought to the USA. The change observed in growth rate show an invasive lag in sika deer population suggesting that sika deer numbers have the potential to expand more rapidly than the current rate. Despite the complicated introduction history and depleted level of genetic diversity, sika deer have proven to be healthy and highly successful deer on the Delmarva Peninsula currently in addition to native white-tailed deer.

I also investigated the competition that exists between the introduced sika deer with our native white-tailed deer, based on dietary resource overlap. A large portion of both species diets were composed of woody browse in the winter, when resources are most limited. Most of the resources used by sika deer (60%) were resources that are part of white-tailed deer diet, and are critical resources for white-tailed deer (>75% white-tailed deer resource use). The degree of dietary overlap measured across several niche size estimators show that sika deer and white-tailed deer significantly (70%) overlap in diet thereby competing for similar resources. The degree of overlap creates a change in white-tailed deer diet, increasing their niche size by 108%, again demonstrating competition over resources. The presence of sika deer on the Delmarva Peninsula also altered the resources that are used by white-tailed deer. The change in resources used by white-tailed deer with sika deer present are of lower quality than those used by white-tailed deer while they are alone. The change in resource use, and the additional pressure from another ungulate on the landscape has the potential to have cascading effects through the trophic levels of the Delmarva, starting with the decline in white-tailed deer numbers. The continued increase in sika deer numbers on the Delmarva is a growing concern for the prolonged success of white-tailed deer and requires additional management considerations.

Chapter 1

A COMPLETE HISTORY OF THE ESTABLISHEMENT OF JAPANESE SIKA DEER ON THE DELMARVA PENINSULA: A 100 YEAR POST INTRODUCTION SUMMARY

Abstract

While many non-native species immediately express their negative qualities which encourage their management (even attempted eradication), some have long lag periods before the population begins to grow out of control. Sika deer have been problematic in many places where they were introduced as novelties or game species through hybridization or aggressive interactions with native deer. In attempt to better manage these species we need to know their native ecosystem. We provide evidence through summarized literature of the manner in which sika deer arrived on Delmarva from Yakushima Island in Japan via a multi-generational stopover in the United Kingdom. We also add morphological and genetic support that confirm the origins and help describe the path of introduction of sika deer to Maryland. We also summarize the growth and change in population size(s) over the last 100 years. This historic understanding is an essential part of coping with the persistent growth of a large, aggressive herbivore that is currently being managed as a game species. We summarize the possible impacts of sika deer including the displacement of native white-tailed deer. The management of this species needs to be carefully observed as they continue to spread throughout the critical saltmarsh of the Delmarva Peninsula.

Introduction

Invasive species are the number one threat to the environment in the 21st century (Didham et al. 2005, Simberloff 2005). Sika deer found on the eastern shores of Maryland and Virginia, and Delaware (here after: Delmarva) are increasing in number and expanding in range. To date, there is a paucity of publications regarding sika deer on Delmarva, and fewer references concerning their introduction to the United States. We conducted a thorough review of literature to determine the history behind the introduction of sika deer, and the implications their introduction may have on the current population. The competitive interactions that this species has with the native white-tailed deer directly affect management decisions of both species, and are confounded by the economic benefits that are associated with having a unique, large game species in the region.

The introduction of sika deer in the United States happened several times (at different locales), with several different subspecies; the first was into Maryland. For a short period after their introduction in 1916, (Flyger 1959, Flyger 1960) there was confusion about which species of deer was actually introduced (Flyger 1960, Presnall 1958). The initial population of 4 or 5 individuals (Flyger 1960) has grown to an estimated 12,000 today (2013: Unpublished data, T. B. Eyler, Maryland State Deer Biologist). Sika deer are a popular game animal but in some areas can also be an overabundant crop pest (Hiroshi et al. 2009, Takatsuki 2009). As the sika deer population continues to grow there are serious concerns about the effects they will have on native species and the habitats they live in (Flyger and Warren 1959, Feldhamer and Armstrong 1993, Takatsuki 2009, Kalb et al. 2013, Chollet et al. 2015). Past sika deer management regulations have either been based on what we know about other native and introduced populations of sika deer from literature, or on regulations that pertain to white-tailed deer.

From Japan to the British Isles

The history behind sika deer in Maryland starts in the early 1800's. A German harbor officer, Jacob Gerhard Gotthold Jamrach took notice of the exotic wildlife that was coming into the Hamburg port. He started a business of buying, selling and showing some of these animals in St. Pauli, Germany. The business was doing so well that it was moved to London, where it was taken over by his son Charles Jamrach in 1841 (Simons 2014). In 1863, the German portion of the business was sold to Carl Hagenbech, who became one of Charles' greatest rivals for acquiring exotic wildlife (Rothfels 2002, Simons 2014), and can be credited with the introduction of sika deer into Germany and Austria in 1893 and 1907 respectively (Pitra et al. 2005) and for providing some individuals to Woburn Abbey (Banwell 2009). Jamrach started a collection, and became known as a "man who could get things" (Robertson 1901). His passion quickly brought him to collecting exotic animals of all types, especially items from Japan which were in high demand due to the Japonism culture (decorating with or in a Japanese style) that had taken hold in England (Simons 2014). The wealthy of England, the United Kingdom, and all of Europe came to know Jamrach as the man to go to for stocking their exotic game farms, parks, and zoos with wildlife. One proprietor of Jamrach's was the 7th Lord Powerscourt, who in 1860 purchased (for around £15 per head [todays value would be about $800 per head]: Robertson 1901, Ratcliffe 1987) 3 female and 1 male Japanese sika deer for his estate in Enniskerry, County Wicklow, Ireland (Powerscourt 1884). This purchase and transport of Japanese deer was a great credit to the abilities of Jamrach when we consider that Japanese ports had just opened to western trade (by threat of force from British, French and US) the same year after having been closed for over 200 years.

The herd in Wicklow increased rapidly and Lord Powerscourt made gifts of the deer throughout Ireland and England (Powerscourt 1884). At the time there were also red deer and sambar deer (*Cervus unicolor*) intermingling with the sika deer. Ratcliffe (1987) questioned the genetic integrity of all stocks which were founded from the herd in Wicklow and suspected hybridization and mxing of genes. One destination of the small Japanese deer was Woburn Abbey in England (still a popular historic site and animal zoo), where the 11th Duke of Bedford (Herbrand Russell: 1858 – 1940) collected several deer species including five subspecies of sika deer (Bedford 1949, Banwell 1995, Swanson and Putman 2009) over several year period from 1893 – 1897 starting with Japanese sika deer (Lowe and Gardiner 1975, Ratcliffe 1987). While the Duke can be credited with the preservation of Peré David's deer which more than likely would have been extirpated without his influence; we also can attribute him with several introductions of non-native deer to other counties (Bedford 1949). The sika deer populations that are now firmly established in both New Zealand and on the Delmarva Peninsula were started from gifts of deer made by the Duke (Banwell 2009, Feldhamer and Demarais 2009). In addition to the missing information regarding the transportation of individuals from Wicklow (by whom and exact numbers and dates), there was a substantial period of time when the multiple subspecies (Banwell 1993, Goodman et al. 2001) of sika deer from the Japanese Islands were all considered to be a single subspecies (Lowe and Gardiner 1975, Ratcliffe 1987) *C. n. nippon*. Feldhamer and Demarais (2009) reference the sika deer in Maryland as the same *C. n. Nippon*, but there is no mention of their recognized number of subspecies.

The 12th Duke of Bedford provided documentation that his father not only attempted on several occasions to create hybrids for specific traits but that he was also somewhat careless with species isolation and on occasion neglected the deer that he lost interest in. In 1941, all of the remaining smaller species of sika deer were lost to disease or culled from the Abbey (Bedford 1949). Morphological and genetic characteristics of the sika deer in New Zealand show that these deer were not established from pure stock, but from some form of sika-sika, or sika-red deer hybrids (Glover 1956, Banwell 1993, 1995, 2009, Swanson and Putman 2009). Glover (1956) also makes reference to the hybridization of sika deer in England, and references the smaller subspecies of sika deer in England in comparison to those of America (suggesting they are of a similar lineage).

From British Isles to the United States

At this time (late 19th and early 20th centuries) in U.S. history there was an extreme shortage of wild game animals, especially on the heavily populated east coast. Market hunting had reduced wild deer populations in Maryland to such low numbers that hunting seasons were closed and restocking efforts were undertaken. The lack of wildlife food sources inspired some (including members of the U.S. Department of Agriculture) to consider game farming, especially deer farming as a source of meat (Lantz 1908, 1910). Sika deer were, and still are, considered a tasty and easily managed species for farming (Powerscourt 1884, Lantz 1910).

Prior to their removal from the Park at Woburn, a gift was made to Clemment Henry in Maryland where he kept 5 sika deer (Flyger 1960, Feldhamer and Demarais 2009). These deer were kept as pets until their release into the wild in 1916 onto James Island, a small island located south of the Choptank River inlet in the

Chesapeake Bay (Figure 1). James Island was 185 acres at the time, however, due to sea level rise and nature weather events, James Island is now split into 3 smaller islands totaling about half the total landmass. In 1920, Dr. Charles Law from Berlin, Maryland purchased 4 or 5 sika deer from a man in Cambridge (most likely Clemment Henry); he kept these and they reproduced. There are no reports on the breeding success of the sika deer on James Island under Mr. Henry, but we can assume that the population increased to some degree because Mr. Law purchased only a portion of the deer that were released to James Island.

A Split in the Maryland Population

Around 1924, a Boy Scout troop purchased up to a dozen deer (presumably the entire stock) from Mr. Law in Berlin, MD and kept them in Ocean City, MD (Figure 1) as a tourist attraction (Flyger 1960). At the time there was an amusement park run by Dan Trimper in Ocean City and he may have been the leader of the same Boy Scout troop (Presnall 1958). As the novelty of these deer wore off, the Boy Scout troop released them across the Ocean City inlet (<300m) to the north end of Assateague Island (Figure 1). The released deer quickly expanded to the entire barrier island, but by 1958 the population was still only 60 – 100 individuals (Presnall 1958, Flyger 1960). Reports from USFWS (2014) report that in 1943 sika deer had dispersed the entire length of Assateague Island and by 1963 their numbers were estimated at 1300.

Population Control

Once established, the population of sika deer in Maryland had several large swings in population size. The first, in 1957 was a result of a large fire on James

Island where the sika deer where initially introduced. The fire destroyed the majority of the understory of James Island, causing the indirect death of sika deer by starvation. Between those killed in the fire and those that died of starvation, more than 160 of these deer perished (Flyger and Bowers 1958, Flyger 1959). Christian et al. (1960) conclude that this large population decline was the result of physiological derangements brought on by high population densities, however, they do not mention the fire or its potential influences on the population. Hayes and Shotts (1959) concluded that the die off was a result of malnutrition and pine oil poisoning as a result of overconsumption of pine without other resources available on the island. Regardless of the cause, the population at the time was between 270 – 300 animals (Flyger and Bowers 1958, Flyger and Warren 1959, Christian et al. 1960) which were primarily concentrated on James Island with a few individuals visiting or establishing permanent home ranges on the mainland, Taylor’s Island.

Harvest of sika deer began on James Island and Taylor’s Island as early as 1938 (Flyger and Warren 1959) but was sporadic through the early 1960’s with only a few harvested annually (Flyger and Davis 1964). Harvest of white-tailed deer resumed, but was limited in the 1930’s through 1960’s. During these years, the few sika deer harvested were taken under a standard, non-specific deer tag (Flyger and Davis 1964, Eyler and Timko 2014). Flyger and Davis (1964) estimated the geographic range of sika deer in the early 1960’s to be about one third of the way across Dorchester County (Figure 1), or about 10 linear miles. Sika deer harvest on Assateague began in 1964 when the population was estimated to have grown to 1,300 (USFWS 2014). Sika deer were so abundant that they had created a clear browse line around the island. The harvest of sika deer helped to control the population which was estimated at 600 deer by 1990 based on a population reconstruction model (USFWS 2014).

As the spread of sika deer continued and the harvest of sika deer increased, sika deer harvests began to be separated from white-tailed deer harvests to collect information for management decisions. Sika deer were distinguished from white-tailed deer as a large game animal in 1973 and harvest regulations actually allowed more sika deer to be harvested than white-tailed deer in Dorchester County (Feldhamer et al. 1978). After sika deer harvest was separated from white-tailed deer harvest, Dorchester County averaged 415 sika deer harvests per year for the first five years (1973–1977: Feldhamer et al. 1978). In comparison, Dorchester County harvested an average of 2,397 sika deer per year in the last five seasons (2009-10 through 2013-14: Eyler and Timko 2010, 2011, 2012, 2013, 2014); almost a 6 fold increase! In addition to the thousands harvested in Dorchester County, sika deer are now harvested annually in Caroline, Somerset, Talbot, Wicomico, and Worchester Counties. Outside of Maryland, sika deer from the same initial introduction in 1916 are harvested in neighboring states (Virginia and Delaware).

Molecular Support

The first study to attempt to determine the ancestral lineage (which of 14 different subspecies) of Maryland's sika deer was not conducted until 2002 (however see Cook et al. 1999 for use in classification). Six sika deer from across the extent of their range at the time were sampled for mitochondrial DNA haplotypes. Dr. Jesus Maldonado from the Smithsonian Institute amplified a section of the control region of mitochondrial DNA. He found all six of the sampled deer shared the same haplotype (and matched 8 sample sequences from Cook et al. 1999) which fit into the *C.n.*

yakushimae cluster which had been very clearly defined in Japanese literature (J. Maldonado, personal communication). The *yakushimae* subspecies of sika deer are the smallest subspecies of sika deer and are native to two small islands in the very south-eastern portion of the Japanese Island chain (Figure 2), Yakushima Island and Kuchinoerabujima Island (Tamate et al. 1998, Nabata et al. 2004, Koda et al. 2008).

We began a more in-depth look into the genetic variation of sika deer on the Delmarva Peninsula to provide insight into historic hybridizations. Recent work with highly variable microsatellite markers demonstrated an extreme lack of polymorphism and heterozygosity in Delmarva sika deer tissue samples. We observed 11 alleles across 10 loci in Assateague samples (N = 29), 14 alleles across 10 loci in Dorchester samples (N = 53), and 28 alleles across 10 loci in samples from Yakushima Island Japan (N = 14). There were more alleles found in the Dorchester samples compared to Assateague samples. This suggests that the transfer of deer from Dorchester to Assateague did not carry all the genetic diversity of the population through the Maryland population split. While there were 12 alleles shared between Maryland and Yakushima, there were also 2 unique alleles in the Dorchester samples that were not observed in Yakushima samples suggesting that not all the genetic information found in Maryland was exclusively from Yakushima sika deer.

A lack of polymorphic enzymes was also observed in sika deer by Feldhamer et al. (1982) who tested between 25 and 40 individuals across 10 enzymes and found no polymorphism or heterozygosity. These data support both the severe bottleneck of sika deer on Delmarva, and the hybridization between several subspecies/species that we know occurred in the UK.

In theory, a lack of genetic variation should cause problems with reproduction and survival due to susceptibility to disease. The sika deer that are on the eastern shore have shown that despite their lack of genetic variation, they have been highly successful, and in some cases shown high resistance to disease and parasitism (Davidson and Crow 1983). Sika deer on Delmarva appear to be well adapted to their environment, but given the way the population has changed over the years since their introduction, this is most likely attributed to sika deer being a generalist herbivore rather than the evolution of the fittest adapting to the new environment.

Physiological Support

Additional information that supports the aforementioned history of transporting sika deer to the United States from Yakushima, Japan by way of the United Kingdom is observed in weights of sika deer on Delmarva. On the native island of Yakushima, body weight for an adult male is about 40 kg (88 lbs.: Takatsuki 1990, Koda et al. 2008). Today, harvest weights of sika deer on Delmarva are comparable to the Yakushima sika deer, between 27 – 37 kg (60 to 80 lbs.: Eyler and Timko 2014) with large adult males reaching 50 kg (110 lbs.) dressed weights (Personal communication, A. Jolicoeur, Farm Manager at Tudor Farms LLC). However, after their initial establishment on the Delmarva Peninsula, there are reports of deer harvested with dressed weights between 58 – 68 kg (125-150 lbs.: Flyger and Warren 1959). These weights are slightly larger than averages that we find in sika deer today, and could reflect some introgression of genes from some of the larger subspecies of sika deer or red deer that were still in the population from their historical lineages in the United Kingdom. These data also suggest that there was more variation in weight of sika deer 50 years ago, prior to the fire of 1957.

Antler characteristics of sika deer on the Delmarva Peninsula also suggest a mixed sika lineage. The antlers of Yakushima sika deer usually have 4 points with a max of 6 (3 x 3: Takatsuki 1990). Larger subspecies of sika deer in the Japanese Islands are known to carry antlers of 8 points (Takatsuki 1990, Bartoš 2009), while mainland sika deer and red deer can commonly carry much larger antlers, 10 – 12 and 12 – 16+ respectively (Bartoš 2009, Winans 1913). Sika deer on the Delmarva Peninsula typically carry 4 – 6 points, but 7 and 8 points are not unheard of (Eyler and Timko 2014). The mixture of larger subspecies genes into the population due to hybridization at Woburn Abbey (Bartoš 2009) could have led to the antler characteristics that we see today on the Delmarva Peninsula.

Implications of the Invader

Now that we understand what lineage the sika deer on the Delmarva are from, it is also critical to understand how they impact their communities (Simberloff et al. 2012). There is no question that the number of sika deer in Maryland has grown and that their geographic range has greatly expanded (Figure 1). Personal communications of observations, harvests, and road kills, show that sika deer are continually progressing (from both Dorchester and Assateague) towards the center of, and north along the peninsula. The continued growth of the population leads to additional concerns, one being the presence of a population of farmed Manchurian (an easily distinguished, and far larger subspecies) sika deer held in captivity in Harrington, DE (approximately 80 km north-east of the Dorchester County). To date, there are no confirmed sightings or harvests of wild sika deer close to Harrington but the chance of further admixture if these captive deer were to interbreed with the wild sika deer is cause for concern. The realistic probability of escapees from the farmed deer breeding with wild type sika deer on Delmarva are still remote, but we predict that this chance will increase as wild sika deer continue to expand northward up the peninsula (sika deer escaping captivity is well documented in several other States: Feldhamer and Demarais 2009). The biological and ecological consequences of such a hybridization could be profound for the Delmarva region. An introgression of genes for larger sika deer, and an increase in allelic diversity could result in an even more adapted species with an existing competitive advantage over native white-tailed deer (Feldhamer and Armstrong 1993, Bartoš 2009).

There are also concerns about the impact of sika deer resource use. Sika deer are a large herbivore with a diet that includes a range of plant species that are found in white-tailed deer diet (Keiper 1985). Shared resource use is thought to create a high degree of competition between the two species. In addition, the impact of sika deer consuming different plants in the habitat is also important and should be investigated. Current sika deer populations are concentrated in both the Chesapeake and Delaware Bay areas. In these areas; they spend time in the salt marsh habitat eating plants that white-tailed deer do not consume, (or consume in very low quantities) that are important parts of this critical ecosystem (Bertness 1999) such as cattail (*Typha* spp.), cord grass (*Spartina* spp.), reed grass (*Phragmites* spp.), sedges (*Carex* spp.) and rushes (*Juncus* spp.) (D. M. Kalb, Unpublished data). The high population ratios (sika deer to white-tailed deer), and population densities of sika deer that exist in some areas cause concern about how their diet on these species is impacting the marshes of Delmarva (Takatsuki 2009).

Since their introduction, scientists and wildlife professionals have been interested in investigating the competition that sika deer have with white-tailed deer

(Flyger and Warren 1959, Keiper 1985, Feldhamer and Armstrong 1993). One of the most obvious and concerning problems of sika deer is the displacement of native white-tailed deer because of their high degree of niche overlap in geographic area, dietary resources, and spatio-temporal activity (Flyger 1959, Keiper 1985,Eyler 2001, Kalb et al. 2013). The direct cause of the change in white-tailed deer range is not fully understood yet, but it is clear that sika deer are taking over some areas. There are portions of Dorchester that prior to 1970 had thriving white-tailed deer communities and annual harvests that now have only sika deer and harvest equivalent numbers (Feldhamer and Demarais 2009).

The implications of invasive deer species can reach beyond their diet use or any direct measure, and may not be realized for several generations (Chollet et al. 2015). Uncontrolled herbivory is already understood to have devastating impacts on the salt marsh community. The periwinkle (*Littoraria irrorata*) was shown to decimate salt marsh grass in the absence of a predator (Silliman and Bertness 2002). Nutria (*Myocastor coypus*) have caused extensive damage due to their herbivory habits both here in Maryland as well as in Louisiana both of which currently have extensive eradication efforts in place (Southwick Associates 2004, Hogue and Mouton 2012). There are few predators of sika deer in Maryland, and none for adult animals other than harvest by humans.

Sika deer on Delmarva provide a unique resource. They are an additional game species to hunters which draws upwards of 5,000 hunters annually (Eyler and Timko 2014). While Maryland’s economy benefits from having sika deer, they are not native and harm the wildlife and the habitats where they are found. Like other exotics on the eastern shore (nutria, mute swan [*Cygnus olor*], and snakehead [*Channa* *argus*]); the presence of sika deer needs to be balanced against the negative effects that result from invasive species. For these others (nutria and snakeheads) the benefit does not appear to influence management and there are state-wide attempts at eradication. There is little hope that even if the desire to eradicate sika deer was accepted (there is little chance of agreement from all stakeholders), that it would even be feasible (Feldhamer and Demarais 2009). Sika deer have already established a foothold in a highly inaccessible portion of the state. The range of sika deer is continually expanding and only time will tell if the changes in harvest regulations (2014-2015 season will increase from 2 sika deer to allow 3 sika deer to be harvested per weapon, no more than one antlered) will curtail this expansion. A more clear understanding of how far (i.e. where sika deer have expanded) to is also necessary. With observations and harvested sika deer coming from counties as far north as Kent MD and Kent DE, regulations need to be implemented that will allow hunters in these northern areas to harvest sika deer, slowing their spread.

Further Investigation and Management

A more clear understanding of the maximum expansion edge of sika deer is necessary. Data from citizen observations, road kills and harvests should be compiled; this will require a multi-state effort. A better understanding of the resource allocation between sika deer and white-tailed deer is essential to the best management of both of these species.

Acknowledgements

We would like to thank the University of Delaware Department of Entomology and Wildlife Ecology for their financial support to the authors while working on this manuscript. We would also like to thank Dr. J. Maldenado, and T. B. Eyler for their contributions of important information that greatly supported this manuscript. We also thank M. Kalb, the associate editor, and two anonymous reviewers for their comments that improved this publication.

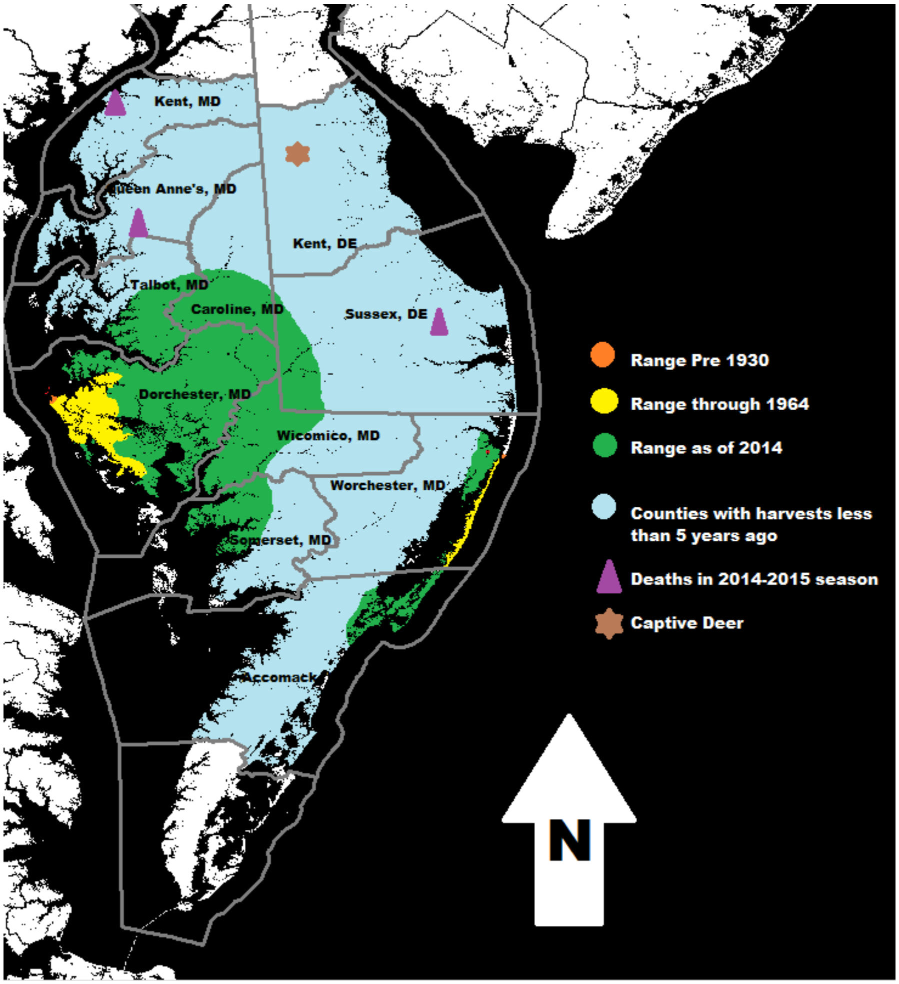

Figure 1 Change in distribution of sika deer on the Delmarva Peninsula over 100 years. Harrington Delaware shows where captive sika deer are held. Islands in red show the location of the initial sika deer release in Maryland (James Islands). Area labeled in yellow marked according to Flyger and Davis 1964. Blue triangles show the location of sika deer deaths known from 2014 season that are considered far outside the current sika deer range.

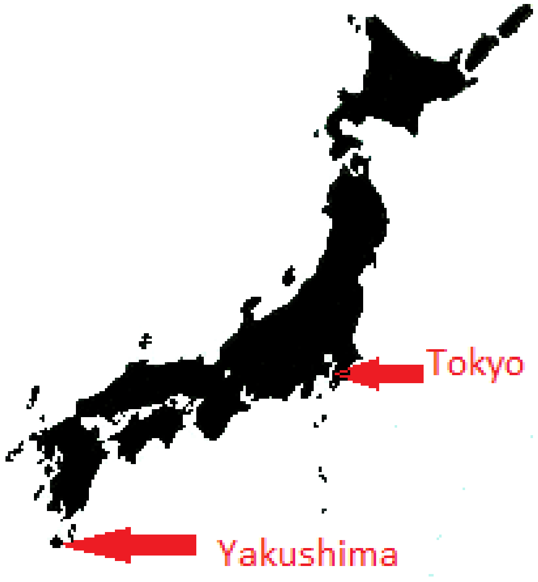

Figure 2 Islands of Japan highlighting the Capital city, Tokyo and the Island of Yakushima, the origin island for the sika deer that were introduced to the UK in the late 1800's and then from there, to the Delmarva Peninsula in 1916.

Chapter 2

GENETIC DIVERSITY AND DEMOGRAPHIC HISTORY OF INTRODUCED SIKA DEER IN MARYLAND AFTER SEVERAL SEVERE FOUNDER EVENTS AND POPULATION BOTTLENECKS

Abstract

Sika deer are native to East Asia, and were introduced to the Delmarva Peninsula in 1916. The introduction and establishment of sika deer to the Delmarva Peninsula is poorly documented, but may have involved ≥1 founder or bottleneck events. We quantified neutral genetic diversity in the introduced population and compared genetic differentiation and diversity to the presumed source population from Yakushima Island, Japan, and a captive population of sika in Harrington, Delaware, known to be of a different origin(s). Based on genetic data from 10 microsatellite DNA loci, we observed reduced genetic variation attributable to founder events, support for historic hybridization events, and evidence that the population did indeed originate from Yakushima Island stocks. Estimates of population structure through Bayesian clustering and demographic history derived from Approximate Bayesian Computation, were consistent with the hypothesized founder history of the Delmarva Peninsula sika deer population in both timing and effective population size (< 5 effective breeding individuals, 36 generations ago). Approximate Bayesian Computation further supported a single introduction of sika deer into the wild before their spread throughout the Delmarva Peninsula. We conclude that free-ranging sika deer on Delmarva are descended from ca. 5 individuals introduced about 100 years ago from captive stocks of sika deer maintained in the United Kingdom. Estimates of

generational gaps fit known dates when we assume 3 – 4 years per generation of sika deer. Free-ranging sika deer on Delmarva have lost neutral diversity due to founder and bottleneck events, yet populations have expanded in recent decades and no evidence of physical abnormalities associated with inbreeding have been documented. We suggest management practices associated with both wild and captive sika deer on the Delmarva Peninsula including increasing harvest areas and specifically managing sika deer outside of Maryland.

Introduction

In the Japanese Islands, sika deer (*Cervus nippon*) were geographically isolated by a series of vicariant events concurrent with cycles of glaciation (Riss-Würm) and changes in sea levels. These events created small island areas with populations maintained by few individuals, which has resulted in isolated subspecies; many populations have low neutral genetic diversity, and are easily separated into clades based on mtDNA lineages (Tamate 2009).

Records are sparse, but Japanese sika deer were apparently released onto James Island in the Chesapeake Bay during 1916, a single introduction of 4 or 5 individuals (Flyger 1960, Feldhamer and Demarais 2009, Kalb et al. 2013: Figure 1). The source population originated in Japan, but spent several generations at Woburn Abbey, England (Feldhamer and Demarais 2009). While in England, the deer were held in captivity with other stocks of unknown origin and allowed to intermingle (Banwell 1999). In 1924, the population of sika deer in Dorchester County, MD was split, and some deer were moved to Assateague Island in Worchester County (Flyger 1960). A severe wildfire in 1957 reduced the population of sika deer on James Island by nearly half (Flyger and Bowers 1958, Flyger 1959). Documentation of the population growth and expansion began during the 1970's. Sika deer are now locally abundant on the southern portion of the Delmarva Peninsula, a region that encompasses Delaware and the eastern coasts of Maryland and Virginia. Despite their founding from so few individuals, sika deer on Delmarva demonstrate remarkable vigor and have experienced near exponential growth over the last century (Davidson and Crow 1983).

The free-ranging sika deer on the Delmarva Peninsula are believed to be geographically isolated into two populations, centering in and around Dorchester County and around Assateague Island (Figure. 1). With no presumed new sources of genetic variation having been introduced since the initial introduction we expect the wild individuals from Dorchester and Assateague to have a similar genetic fingerprint (with minimal affects associated to genetic drift within 100 years).

Introduced populations often experience dramatic changes in effective population size and geographic range, with implications for the maintenance of genetic diversity (Hunter and Gibbs 2007, Dlugosch and Parker 2008). Historically, translocations were not well monitored, or detailed in records. Additionally, introductions by private citizens are often clandestine. While we only have evidence of a single introduction through multiple founder events, an investigation of genetic diversity and demographic history will provide evidence to better understand sika deer on the Delmarva Peninsula.

The introduced wild sika deer population offers a unique opportunity to investigate the effects of serial founder events on neutral genetic diversity (Sakai et al. 2001). The genetic profile of introduced sika deer population will provide insight into timing of their introduction, bottlenecks, number of founder individuals, historic ranges from which the introduction was established as well as provide insight into their evolution and adaptation (Menotti-Raymond and O'Brien 1993, Sakai et al. 2001, Lawson Handley et al. 2011). Low genetic diversity can have implications on the success of isolated populations such as decreased fitness, and limiting a population's ability to naturally adapt to changes in their environment (Reed and Frankham 2003, Baalsrud 2011). Sika deer are a valued game species in Maryland and the possible effects of low genetic variability will impact long term management of this species.

The presence of sika deer on the Delmarva Peninsula is exaggerated by a herd of captive individuals held in Harrington, DE. The captive sika deer are held within several high fenced enclosures and provide an excellent outgroup for genetic comparisons. Genetic analysis should allow confirmation that there have been no escapees, and while there have been no known escapees, the implications of such an event needs to be addressed due to the presence of the wild type, their non-native status, and the known escape potential of sika deer (Feldhamer and Demarias 2009). Wild sika deer on the Delmarva Peninsula are reproductively isolated from each other, captive sika deer on the Peninsula, and from other congeneric species (e.g. elk [*Cervus elephus*]).

The overall goal of this study was to quantify genetic diversity in the wild sika deer on the Delmarva Peninsula. We compared the genetic diversity in free-ranging sika deer populations to the putative source population, as well as a population of captive sika deer on the Delmarva Peninsula. An analysis of the neutral genetic fingerprint of all sika deer on the Delmarva Peninsula will allow us to better understand how sika deer spread across the Peninsula. We also modeled the demographic history of the wild sika deer on the Delmarva Peninsula to provide additional information about timing of, and size of founder events. Finally, we discuss mechanisms that lead to the success of populations from small founder events.

Sample Areas

We collected tissue or fecal samples from sika deer from 4 geographically isolated locations; Assateague Island, MD; Dorchester County, MD; captive deer in Harrington, DE (~80 km northeast of Dorchester County: Figure 1); and Yakushima Island, Japan the presumed source population for wild sika deer on the Delmarva Peninsula (Figure 2). Samples from Dorchester County and Assateague Island were collected from hunter-harvested deer throughout the areas on both public and private lands. Both Dorchester and Assateague Island are comprised of salt marsh and mixed woody wetland habitats that hold sika deer in varying numbers. Dorchester has areas that are dominated by sika deer in the southern and western portions of the county, as well as areas that are dominated by native white-tailed deer (*Odocoileus virginianus*). White-tailed deer have declined throughout Assateague Island, and sika deer have become the primary cervid species.

Captive sika deer are on the Delmarva Peninsula are kept in a fenced enclosure. These deer are known to be of a different provenance than the wild deer on the Delmarva Peninsula. These captive sika deer are an admixture from several different game farms and are expected to have a different allelic diversity than the wild sika deer, which will allow us to use these deer as a comparative outgroup. To the best of anyone's knowledge, no sika deer from this facility have ever escaped, and no sika deer from the wild have encountered the captive facility. Because of the different demographic histories we expected to find greater allelic diversity and more private alleles within the captive sika deer andYakushima, Japanese samples.

Methods

We collected samples from 55 sika deer harvested throughout Dorchester County, and 30 sika deer harvested on Assateague Island during the 2012-2013 hunting seasons (Table 1). We collected a 3 cm cube of liver or muscle tissue from harvested deer. Since harvests occurred over many congruent days in several locations, we accepted the help of hunters and managers to collect samples and data from their properties. We provided each collector detailed instructions on how to collect tissue and data.

We collected fecal samples from 12 sika deer held in captivity in Harrington, DE (Table 1, Figure 1). We collected 6 fecal samples from an all-male pen, and 6 from an all-female pen. We collected only fecal samples that were fresh (e.g., pellets were wet, shiny and covered in a thick mucosal layer) or where we witnessed defecation.

We placed all samples into sterile 50-ml centrifuge tubes containing 25 ml of 95% ethanol (enough to cover the sample); we changed gloves between samples. For all samples, we recorded the collection date, harvest location, sex of deer and species of deer on the 50ml tube as well as on paper with pencil inside the tube. We stored all samples in a dark cabinet until we conducted DNA extraction. We extracted DNA from samples with either Qiagen (Venlo, Limburg, Netherlands) or Bioline's (London, UK) extraction kit for the appropriate sample type (i.e. Blood and Tissue DNAeasy kit for tissue samples, or stool kit for fecal samples), and according to the manufacturer's instructions for maximum quantity yield.

We also used 14 DNA samples that we received from the Yakushima Island sika deer population, Japan (Table 1, Figure 2). These samples were sent to us from Dr. H. Tamate and associates from Yamagata University. Samples were sent as desalted pellets and were re-suspended and diluted to a concentration of 100ng/µL. We stored all DNA at -80°C until analysis.

Identification of species

On Assateague Island and in Dorchester County, sika deer and white-tailed deer are sympatric and hunting seasons are concurrent. To confirm that the samples collected were sika deer, we performed a restriction fragment length polymorphism (RFLP) assay for each sample (n = 109: Appendix Table 16).

We identified a set of primers that amplifies a section of the mitochondrial D-loop, resulting in a fragment of ~464bp in length for both sika deer (Wolf et al. 1999) and white-tailed deer. We identified differences in base-pair composition of the sequences using the National Center for Biotechnology Information (NCBI: GenBank) for both Yakushima sika deer and white-tailed deer. We slightly modified the light strand primer from Wolf et al. (1999), L14735, at 2 separate base pair point changes: transitions at position 9 C to T, and position 16 T to C. Our modifications to Wolf et al. (1999) produced a better match to both Yakushima sika deer and white-tailed deer genomes (sika deer: Wada et al. 2007; white-tailed deer: Seabury et al. 2011).

5’: AAA AAC CAT CGT TGT CAT TCA ACT A

The heavy strand primer (H15149) differed in two positions for sika deer and one position for white-tailed deer (Wada et al. 2007, Seabury et al. 2011), all at unique locations, and therefore we did not modify it. We amplified this fragment using the polymerase chain reaction (PCR), with an initial 3-min denaturation at 96 °C, followed by 45 cycles (96 °C for 30 s, 60 °C for 1 min) and final extension at 72 °C for 3 h (Wolf et al. 1999). The reaction mix included 5µg of each primer (Invitrogen; Carlsbad, CA), 0.05µg BSA (Promega;Madison, WI), 12.5µL MyTaq Red Mix (Bioline), about 50 ng DNA template, and enough DI water to bring the final volume up to 25 µL.

Based on the sequence comparisons (Wada et al. 2007, Seabury et al. 2011), we selected restriction enzyme HinF1, which cut fragments that were large enough to be viewed easily on an agarose gel. HinF1 cut G┃AN(R=[A])TC in white-tailed deer, resulting in two fragments (198bp and 265bp), but did not cut the sika deer fragment (Figure 3). Our restriction cocktail consisted of 5 units of HinF1, 0.025µg BSA (Promega), 2µL RE 10X Buffer (Promega: included with enzyme), 5 µL of the PCR products, and was brought to a final volume of 10 µL with DI water. We digested the samples at 37°C for 3 hours, then held them at 4°C. We electrophoresed the products on a 2.0% agarose gel containing ethidium bromide (5µL per 100 µL of agarose mix) and visualized results under UV light.

Genetic diversity and population structure

We evaluated 16 microsatellite DNA loci developed for cattle (*Bos taurus*), sheep (*Ovis aries*), or reindeer (*Rangifer tarandus*) that successfully amplified in multiple species of cervids, and displayed high polymorphism and low genotyping error rates (Table 2 & Appendix Table 17). Due to low-quality results in preliminary analysis, we omitted six of the loci and used the remaining 10 for all samples. We carried out amplifications as follows: 4 min denature at 96°C followed by 35 cycles of (30 sec denature at 96°C, 60 sec annealing (specific temperatures listed in Table 2), 90 sec extension at 72°C), and a final extension of 1 hour at 72°C before being held at

4°C. We created our PCR reaction mix using 10µl PCR Master Mix (Promega[Madison, WI]: 400µM each dNTP; 3mM $MgCl_2$), 5µM of both forward and reverse primers, 0.05µg BSA (Promega), 50ng template DNA and enough DI water to bring the final volume to 20µL per sample. We amplified all loci individually and combined them post-PCR for fragment analysis. We used primers fluorescently labeled at the 5' end with Applied BioSystems (Carlsbad, CA) G5 filter set (see Table 2 for dyes) and ran the samples on ABI 3730 DNA analyzer at the Delaware Biotechnical Institute (DBI). We scored alleles using Genemapper Software 3.7 from Applied BioSystems (2004). We only scored peaks in Genemapper that were 100 relative fluorescence units (RFU's) or greater. Low allele peaks (<300 RFU's) were not common and we only accepted them when they were alleles that were already common in the population (following a relative threshold of calling: Whitlock et al. 2008). We scored heterozygous alleles when the second peak was within 50% of the primary peak's RFU size. All RFU allele like peaks that fell within the expected range for the loci but were less than 3X the background noise were scored as missing.

Null alleles may bias downstream genetic analyses (Dabrowski et al. 2014), and heterologous markers have a greater potential for null alleles. Therefore, we estimated null allele frequencies and mean error rates via Dempster's EM method (Dempster et al. 1977) with the program GENEPOP (Raymond and Rousset 1995, Rousset 2008).

We indexed genetic variation among populations with allelic richness and observed heterozygosity at all loci. We compared the observed levels of heterozygosity to expected values by testing for Hardy-Weinberg equilibrium using the program GENEPOP (Raymond and Rousset 1995, Rousset 2008). For all loci that had 4 alleles or fewer per population, we used the Fisher's exact test. For locus OarFCB193, we used a Markov-chain method at 100 batches and 1,000 iterations per batch. We determined significance for both tests at the 0.05 level (Goodman et al. 2001).

The number of observed alleles in a population is influenced by sample size. Since our sample sizes from each area were different, we quantified genetic diversity, and estimated private alleles using a rarefaction procedure in the program HP-Rare (Kalinowski 2004, Kalinowski 2005) in each population.

We used an analysis of molecular variation (AMOVA) based on Wright's F statistics (F_{ST}, F_{IT}, F_{IS}) implemented in the program GenAlEx (Wright 1965, Peakall and Smouse 2006, 2012). Measurements of F statistics were calculated across all 10 loci and statistical significance was assessed based on 999 permutations among individuals or populations, as appropriate. We also estimated genetic diversity within individuals, within populations, between populations, and between geographical regions with GeneAlEx.

We estimated population structure of all sika deer on the Delmarva Peninsula using the samples from Japan and captive deer as outgroups. We performed a principle coordinate analysis (PCoA) to compare genetic similarity of individuals within and among populations. We performed the PCoA on matrices of genetic distances between individuals based on a converted covariance matrix using the computer program GeneAlEx (Peakall and Smouse 2006, 2012). We also estimated population structure with a Bayesian clustering analysis performed in the computer program STRUCTURE (Prichard et al. 2000). We used a burn-in of 100,000 Markov Chain Monte Carlo reps followed by 1,000,000 iterations. We estimated clusters (K) from 1 through 7, with 8 repetitions of each K. We determined the best fit cluster solution using a slight modification of the Evanno method (Evanno et al. 2005) in the computer program STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Demographic history

We used approximate Bayesian computation (ABC) to make inferences about wild sika deer on the Delmarva Peninsula including the potential for serial founder events, using the computer program DIYABC v 2.0.4 (Cornuet et al. 2008). approximate Bayesian computation analysis can model complex population histories, including bottlenecks or founder events,, changes in population sizes, and admixture. We quantified support for demographic scenarios by generating simulated posterior probability models based on given demographic priors in a coalescent framework (Lawson Handley et al. 2011, Cornuet et al. 2008). We selected uniform probabilities for all scenarios on all parameters.

All priors provided in DIYABC scenarios were based on the best information available regarding historic and current status of wild sika deer on the Delmarva Peninsula and Yakushima Island, Japan including a proportional buffer (Table 3). We estimated current effective population sizes based on total population estimates for Dorchester, Assateague, and Yakushima Japan: we assume that Yakushima, Japan samples represents the allelic diversity of the founding stocks (Table 3). The DIYABC output includes estimates of all parameters for timing (in generations: T_i), duration of bottlenecks (in generations: db_i) and population sizes after bottlenecks or founder event (Nf_i).

For each ABC analysis we created 1 million simulated data sets per scenario, using the same number of loci and individuals as the original data set. We ran all mutation rate (μ) models with prior distributions for a generalized stepwise mutation model (GSM) for each locus (uniform mean mutation rate: 1.00E -004 min, 1.00E -3 max; uniform coefficient P: 1.00E -001 min, 3.00E -001 max; gamma individual mutation rate: 1.00E -005 min, 1.00E -002 max; gamma individual coefficient P: 1.00E -002 min, 9.00E -001 max). We calculated the relative confidence in each set of scenarios via polychotomous logistic regression using the best 0.1 proportion of the data sets simulated. We calculated posterior distributions from this top 0.1 proportion of the data from the best scenario using linear regression of the logit transformed results (Cornuet et al. 2008).

We considered a range of demographic scenarios in varying complexity, from simple independent introductions without admixture and a single founder event (3 historic events), to introductions from admixed populations with multiple founder and bottleneck events (9 historic events and 19 separate parameters). All of our demographic models included a split between the two wild portions of Delmarva sika deer, Dorchester and Assateague (timing and length of founder event changed), all involved a bottleneck in the Dorchester population, and all involved these two populations splitting after the stock was founded from the Yakushima Japanese population. We compared scenarios in groups of 2 – 4, changing single parameter events (e.g., did bottleneck happen at the same time period as the introduction or were there two separate events) and compared the best scenario from each for a final best case estimation of timing and magnitude of demographic events.

Our simplest scenario was wild Delmarva Peninsula sika deer were founded directly from the Yakushima, Japan population: a population of sika deer that split into modern Yakushima Japan samples and a second branch (wild Delmarva Peninsula sika deer) that later split into Dorchester and Assateague sika deer. The bottleneck in the Dorchester population happens after the split from Assateague deer in this scenario. Our second scenario was that wild Delmarva Peninsula sika deer are from a different stock of sika deer, but incorporated some genetic admixture with sika deer from Yakushima, Japan; a population of sika deer that splits forming an "unknown" population, and the Yakushima, Japan population. Individuals from these populations meet and their offspring are a combination of the genetic lineages of both. The third scenario involves the Yakushima, Japan sika deer as the parent lineage to wild Delmarva Peninsula sika deer, but there was introgression of genes from another stock in the recent past; the Yakushima, Japan sika population split from an unknown population, followed by a series of founder events. Individuals from a branch of the Yakushima Island line meet some from the unknown line and this new lineage forms the base of the branch for the Introduced wild Delmarva Peninsula sika deer.

To verify the performance of the selected model, we used the model check option in DIYABC to estimate the goodness of fit of the simulated data sets with our original data (Cornuet et al. 2014). Model check was based on 18 summary statistics across all three original populations including: mean number of alleles, allele size, F_{ST}, mean genetic diversity, shared allele distance, and $(\delta\mu)^2$, a measure of genetic distance between populations. We also estimated our confidence in the selected model using linear discriminant analysis of the summary statistics to provide a confidence interval to differentiate between sets of scenarios (Cornuet et al. 2014).

Results

Because all wild Delmarva Peninsula samples were collected from harvested sika deer, we know that each was from a unique individual. Based on microsatellite results we were able to identify each of the captive sika deer as a unique individual. All of the sika deer samples from Yakushima Japan were collected from unique individuals.

All but 2 of our samples were identified as sika through RFLP analysis; 1 from each Dorchester and Assateague identified as white-tailed deer or did not amplify. We genotyped 109 total samples; 12 captive sika deer, 14 Yakushima Japan, 29 wild sika deer from Assateague Island, and 54 wild sika deer from Dorchester County. We observed 41 alleles across the 10 loci, with more alleles in both the Yakushima, Japan and captive sika deer samples than in either of the wild Delmarva peninsula populations (Assateague and Dorchester). We randomly selected and repeated samples to re-genotype and scored them separately (blind error rate: Bonin et al. 2004). . The error rate for scored genotypes was 4.6% based on 283 repeated loci out of 1409 scored loci. Our estimated error rate (program GENEPOP) was 4.9% based on population-locus combinations with more than 3 alleles per locus. Our genotypic data set was 93% complete across all 10 loci, with 48% of missing genotypes observed at locus BM1225.

Allelic diversity ranged from 1 allele (OarFCB304) to 8 alleles (OarFCB193) between populations with a mean of 4 alleles per population (Table 2). Most of the observed allelic diversity was within captive sika deer and Yakushima Japanese samples (29 of 41 observed alleles). We observed 2 private alleles, one at each marker, IGF-1, and BM203 in the wild Delmarva Peninsula sika deer, both of these were found in the Dorchester samples. No private alleles were observed in Assateague samples. The Yakushima Japan samples had 11 private alleles and captive sika deer samples had 10 private alleles (Table 4).

Expected and observed heterozygosity deviated from Hardy-Weinberg equilibrium expectations in 5 population-locus combinations (Table 5). Both BM1225 and OarFCB193 displayed a deficit of heterozygotes in the Yakushima Japanese samples, RT27 was heterozygote-deficient in the captive sika deer samples, and IGF-1 was heterozygote-deficient in the wild Dorchester samples. One locus, OBCAM, displayed an excess of heterozygotes in the wild Dorchester population (Table 5). Mean null allele frequency was 0.13 for estimated locus-population combinations (n=9).

Population Structure

The AMOVA showed all populations were differentiated from each other based on F_{ST} (Table 6), F_{IS} and F_{IT} (Table 7). Most (54%) of the genetic variation of these populations was measured (by F_{ST}) among populations (Figure 4). In our PCoA analysis, the top two axis explained 51% of the cumulative variation between populations (Figure 5). Wild sika deer (Assateague and Dorchester) samples produced overlapping plots. Sika deer from Yakushima, Japan overlapped with both wild sika deer samples but were further dispersed. Samples from captive sika deer clustered together away from the other samples with very little overlap (Figure 5). Bayesian clustering analysis supported two clusters. Captive sika deer samples were clearly differentiated, while the wild sika deer (Dorchester and Assateague) populations formed a separate cluster; the Yakushima Japan samples fit into both clusters (Figure 6, Figure 7).

Demographic history

We had 3 top model scenarios in our approximate Bayesian computation (Figure 8). Comparing and pre-evaluation of scenarios selected our third model 86% of Direct approach estimates and 97% of logistic approach estimates. The most plausible scenario involved 7 time stages. Our top model included a ghost population with genetic admixture between time periods 4 and 6 (when sika deer where in the United Kingdom[England and Ireland]). Estimates of posterior population statistics are summarized in Table 3 including known values from historic literature. Bottleneck durations were long in the US, 5.7 (db), 6.1 (db2), but short while in the UK 2.1 (db3) generations.

We estimated that (in reverse order from current date) 28.3 (Nf1) effective breeders survived the fire on James Island which occurred 13.9 (t1) generations ago. The actual year of the fire on James Island was 1957, or 58 years before present, a mean generation time of 4.2 years. The split of the wild Delmarva Peninsula population, from Dorchester to Assateague, was estimated at 21.7 (t2) generations ago. The actual year of the split between Dorchester and Assateague was 1924, a mean generation time of 4.2 years per generation. Our estimate for founder individuals was 3.6 (Nf2), which was estimated at 35.8 generations ago (t3). The introduction of wild sika deer occurred in 1916, about 2.8 years per generation. Sika deer arrived in England about 45.3 (t4) generations ago from two populations, the ghost population of 11 effective breeders (NS) and Japan deer (Nf3), with 3.4 effective breeding individuals. The sika deer that arrived in England went through a bottleneck on their way from Ireland, an estimated at 74.6 generations ago (t5), in 1884. The sika deer populations that were in England and Ireland at this time derived from several subspecies and mixed stocks; we estimated a coalescence date of 4700 (t6) generations ago. In our model checking, only three of the 18 statistical parameters had simulated data that were less than the observed values, 2 mean number of alleles (in Assateague and Yakushima Japan simulated populations), and 1 mean size variance. The goodness-of-fit confidence in our selected scenario was high: of 500 simulated scenario estimates; 7 supported scenario A, 3 supported scenario B and the remainder, 98%, supported our top model scenario C (Figure 8).

Discussion

The amount of genetic diversity maintained through founder events is influenced by the number of breeding individuals, drift and other chance events, and the rate of population growth post-introduction (Estoup et al. 2001). Sika deer on the Delmarva Peninsula were founded by few individuals, from source stocks with limited diversity. The introduced population experienced slow growth and post-introduction bottlenecks, resulting in populations with low neutral genetic diversity.

Sika deer on many Japanese Islands have lower genetic variation than observed in mainland populations of cervids (Lü et al. 2006). The colonization of Japan by sika deer through the rise and fall of sea levels during the Riss-Würm (North American equivalent: Sangamon) interglacial and Würm (North American equivalent: Wisconsinan) glacial periods of the Pleistocene produced small, isolated populations of sika deer (Tamate and Tsuchiya 1995). These small populations display limited variation within, and genetic differentiation between populations (Nagata et al. 1998*b*, Goodman et al. 2001, Tamate 2009). These geographic separations translated into populations on the Delmarva Peninsula (wild vs captive) that are both physically and genetically very different.

We observed more alleles, and a greater number of private alleles in the captive sika deer samples compared to both the Yakushima, Japan samples and wild Delmarva Peninsula sika deer samples (Table 4). The high proportion of private alleles found between Yakushima Japan and captive sika deer samples is likely a result of the historic separation between mainland and Japanese sika deer, and because the captive sika deer are a mixture of different sika deer stocks (Olson et al. 2013).

We observed a decline in genetic variation in populations congruently with the timing and pattern of establishment. In an increasing order of genetic variation, wild sika deer on Assateague was founded from wild deer in Dorchester, founded from stocks in England and Ireland, which were derived from Japanese sika deer (Nagata et al. 1998*a*, Goodman et al. 2001, Senn 2009). Samples genotyped in England averaged between 0.13 – 0.38 heterozygosity values, and 1.44 – 2.33 alleles per locus with similar allele frequencies at individual locus (Senn 2009; Supplemental Table 18). Nagata et al. (1998*a*) observed three loci across two populations and had an average observed heterozygosity from 0.21 – 0.23, with 1.67 – 3.67 alleles per locus. Our samples from wild sika deer on the Delmarva Peninsula averaged an observed heterozygosity of 0.05 – 0.12 and 1.1 to 1.4 alleles per locus for Assateague and Dorchester respectively.

While most of the alleles that we observed in wild Delmarva Peninsula sika deer populations were found in the Yakushima Japan samples (12 of 14), there were two private alleles. These data support three hypotheses about the wild sika deer on the Delmarva Peninsula. The first is that the primary contributing population is the Yakushima Island subspecies (*C. n. yakushimae*). The second is that there may have been genetic admixture with other stocks prior to the introduction onto the Delmarva

Peninsula. Antecedent sika deer to the wild Delmarva Peninsula from Ireland and England had the chance to interbreed with multiple other deer species and subspecies (Bedford 1949). Alternatively, the alleles may exist in Yakushima but were not present in this sample. The private alleles may have been lost from Yakushima but retained in Delmarva, which seems unlikely because more variation should be lost from the Delmarva deer during the multiple founding events and bottlenecks. It is also possible that the alleles are novel and derived from mutation after introduction. Finally, the low allelic diversity and proportion of shared alleles observed in both wild Delmarva Peninsula (Assateague and Dorchester) sika deer populations and the source stock in Yakushima Japan is consistant with a single introduction and subsequent founder event.

Genetic variation we observed in Delmarva Peninsula sika deer is similar to other populations of ungulates that have been founded from few individuals. For instance, introduced populations of elk in Pennsylvania and white-tailed deer in Finland display reduced variation relative to the source stocks (Williams et al. 2002, Kekkonen et al. 2012). Elk in Pennsylvania showed 7 of 10 loci were fixed or had been reduced to 2 alleles (Williams et al. 2002). White-tailed deer introduced to Finland maintained greater allelic richness and higher heterozygosity (5.36, 0.692) across 14 loci than observed in sika deer and elk; however, they were founded from deer that were more highly variable (Kekkonen et al. 2012). The population in Finland also received additional genetic variation from a secondary introduction 14 years post founding, and grew rapidly providing additional chance for genetic drift (Kekkonen et al. 2012, Brommer et al. 2015). Similar to the translocation of bighorn sheep (*Ovis canadensis*), subsequent establishments of sika deer in the wild populations of the Delmarva Peninsula show progressive decline in neutral genetic variation (Olson et al. 2013). Wild sika deer of the Delmarva Peninsula were founded from a single introduction of stocks with low diversity, followed by a lag in population growth and at least 1 bottleneck event. Additionally, since the population of sika deer on the Delmarva Peninsula remained small for several generations, there was a potential for the loss of neutral genetic variation due to genetic drift.

Genetic variation is correlated with reproduction, as accumulated inbreeding leads to reduced sperm count and decreases in birth rates or juvenile survival) and increased susceptibility to disease (O'Brien et al. 1985, Sakai et al. 2001, Lawson Handley et al. 2011). However, wild Delmarva Peninsula sika deer have proliferated and in some cases replaced native white-tailed deer. Wild sika deer have lower susceptibility to parasites than native white-tailed deer (Davidson and Crow 1983) in spite of their lack in genetic diversity. Therefore, despite low neutral diversity, sika populations appear to be robust. In some cases, adaptive diversity can be maintained if the forces of selection outpace genetic drift, as has been observed for some loci in the immune system (Hedrick 2004). It is possible that introduced sika have retained sufficient adaptive variation to be successful in their new environment.

Limited genetic diversity precludes fine-scale inferences on population substructure and assignment on the Delmarva Peninsula. We calculated high values (Wright 1978, Balloux and Lugon-Moulin 2002) for F_{ST}, and F_{IT} as a result of the complex nature of the introduced wild Delmarva Peninsula populations. The calculated F_{IS} values were not as high (Table 7), but do suggest some degree of population structure within the sampling area due to social behavior or likely geography. The assignment into population clusters also confirms a lack of gene flow between captive sika deer and the wild sika deer of the Delmarva Peninsula. The Yakushima Japanese samples were found to have mixed population assignment with some individuals more similar to captive sika deer samples and some individuals more similar to the wild sika deer samples.

Approximate Bayesian computation supported the recorded history of the introduction of sika deer to the Delmarva Peninsula. The best-fit demographic scenario for our computational analysis involves a single introduction of sika deer forming the wild population. The rate of genetic admixture in our best-fit scenario suggests that there was little introgression of new genes in sika deer that were in the United Kingdom. The duration of bottleneck events were much longer in the US (Table 3: db and db2 vs db3) than in England or in Ireland. This further supports how wild sika deer on the Delmarva Peninsula generally went unnoticed in the decades post-introduction. On the contrary, sika deer introduced to Ireland expanded quickly (Powerscourt 1884). In the US, the range and population size of sika deer was most likely restricted from an invasive lag.

Generation estimates from ABC follow a similar growth rate estimated in bottleneck durations. The average years per generation were also less in the UK (2.7) than were estimated in the US (4.2) based on known dates (Table 3: t1 and t2 vs t3 and t4) but were consistent between regions. Longer periods of time with small population size increase the effects of random drift on the population, and could have resulted in the loss of some neutral genetic variation. Until recently, the expansion of wild sika deer northward up the Delmarva Peninsula has been fairly limited.

From an original introduction of 5 individuals, the population of wild sika deer of the Delmarva Peninsula has increased to an estimated 10,000 - 12,000 and has spread through 7 counties, as well as into neighboring Delaware and Virginia. The spread of sika deer across Delmarva continues with new sightings and reports of harvests even farther north into Maryland and Delaware nearly every year. The Maryland Department of Natural Resources has tried to manage sika deer as a local economic and social benefit to the communities of Dorchester and Worchester. Recently, harvest bag limits were increased for the 2014-2015 hunting season, and harvest management will be the primary means of controlling sika deer.

In addition to these regulation changes, we strongly suggest that sika deer become legal game in Queen Anne's, Kent and Cecil Counties of Maryland. Allowing hunters to harvest deer as they move northward may help in preventing further establishment. We also encourage the Delaware Department of Natural Resources to design and implement management protocols specifically for sika deer, which should include liberal harvest regulations and ample administration of permits for agricultural damage (ideally to prevent sika deer spread through the state). Most importantly, Delaware officials will need to address the future of captive sika deer on the Delmarva Peninsula, and if the potential risks associated with an escapee or released animal are warranted.

For future research we suggest using a wider set of microsatellite loci and evaluating single nucleotide polymorphisms, which would be informative in addressing if the sika deer of the Delmarva Peninsula have evolved or maintained any adaptive diversity during their founding. We also suggest a more thorough sampling of sika deer on the Delmarva, especially in Wicomico, MD, and Sussex, DE to evaluate the spread of sika deer as individuals from the original Dorchester and Assateague populations begin to meet again.

Acknowledgements

We would like to thank the University of Delaware Department of Entomology and Wildlife Ecology for their financial support to the authors while working on this manuscript. We would also like to thank Dr. H. Tamate from Yamagata University and associates for the kind donation of sika deer DNA samples from Yakushima Island. We thank Dr. M. Achenbach for information and access to his deer pens. We thank Tudor farms LLC & P. Jones (including A. Jolicoeur and K. Compton); Biologists at Assateague National Sea Shore; and Biologists at Blackwater National Wildlife Refuge for access and assistance in collecting samples.

Table 1 Summary of sampling regions within areas of described locations and the total number of samples collected in each. Counts of samples that were included from each group in each type of analysis are also listed.

Region	Location	Samples	Count & Type	mtDNA species determination	SSR Analysis
Japan	**Japan**	**Yakushima**	14 Tissue	14	14
Delmarva	**Delaware**	**Delaware**	12 Fecal	12	12
Delmarva	**Maryland**	**Dorchester**	55 Tissue	54	54
Delmarva	**Maryland**	**Assateague**	30 Tissue	29	29

Table 2 Microsatellites used to determine population structure and genetic variation of sika deer on the Delmarva Peninsula with total number of alleles observed from all populations, the dye and colors, annealing temperatures for amplification, and grouping of markers for reading.

Locus	**Total Alleles**	**Dye/color**	**Annealing Temp**	**Group**
OarFCB193	8	NED-Yellow	58	1
OarFCB304	1	VIC-Green	58	2
RT27	3	PET- Red	55	2
OBCAM	3	NED-Yellow	55	2
BM4006	3	VIC-Green	54	1
BM6438	5	6FAM- Blue	59	1
BM1225	4	PET- Red	56	3
BM4107	5	6FAM- Blue	54.5	3
BM203	4	6FAM- Blue	56	2
IGF-1	4	NED-Yellow	56	3
RM188	NA	VIC-Green	54	NA
TGLA126	2	6FAM-Blue	56	NA
TGLA127	1	NED-Yellow	54	NA
TGLA337	NA	PET-Red	?	NA
IDVGA55	1	NED-Yellow	56	NA

Table 3 Parameters used as priors for approximate Bayesian computation with known information. . Effective population sizes were estimates as between 10 and 25% (Palstra and Fraser 2012) of total population estimates when provided unless otherwise noted. Resulting Posterior estimates shown as means, medians and 95% and 5% bounds.

		Prior information used for approximate Bayesian computation analysis			Resulting Posterior estimates from ABC			
Event	Parameter	Information	Prior	Citation	mean	median	q050	q950
Ne Dorchester	Dorchester	Annual harvest is 2500 for Dorchester alone. Population estimate for Maryland is ~10,000-12,000 with the bulk on individuals in the Dorchester population.	250 - 2500	Eyler and Timko 2014	1300.0	1230.0	414.0	2340.0
Ne Assateague	Assateague	See above	80 - 1125	USFWS 2014	156.0	111.0	83.5	403.0
Ne Yakushima Japan	Japan	Population between 2,000 and 18,000 (Ne:200 - 4500)	200-5,000	Tsujino et al. 2004	3070.0	3200.0	952.0	4840.0
Ne Ghost	Ns	Number of deer that contributed to admixture while in UK.	2 - 20 ind.	No account provided	11.0	11.1	3.0	19.0
Fire ‘57	t1	1957 (59ybp)	10 - 30 gen.	Flyger and Bowers 1958	13.9	12.7	10.0	21.8

Fire Lag length	Db	population remained small for several generations	2 - 7 gen.	Presnall 1958	5.7	6.3	2.0	7.0
Fire Ne	Nf1	160 total deer (Ne: 16 - 40)	14 – 45	Flyger and Bowers 1958	28.3	27.7	15.1	43.1
Assateague split	t2	Assateague founded. 1924 (91ybp)	15 - 45 gen.	Flyger 1960	21.7	20.3	15.4	33.4
MD founding	t3	Arrival/ release into wild. 1916 (99ybp)	16 - 50 gen.	Flyger 1960	35.8	36.0	22.7	47.7
MD lag	db2	Uncertainty about time between actual arrival and release into wild. Population remained small for several generations	2 - 10 gen.	Presnall 1958	6.1	6.1	2.0	10.0
MD founders	Nf2	4 or 5 individuals * (such a small founding population we allow for the entire count)	1 - 5 ind.	Flyger 1960	3.6	4.3	1.0	5.0
UK Woburn Abbey	t4	Arrival in England 1893 (123ybp)	20 - 61 gen.	Bedford 1949, Lowe and Gardiner 1975	45.3	45.9	29.4	58.7
Admixture	r1	Some degree of admixture, degree relatively unknown.	0.001 - 0.3%	Bedford 1949, Banwell 1999	0.138	0.132	0.013	0.282
Ireland Founding	t5	Arrival in UK (Ireland) 1860 (155ybp)	26 - 78 gen.	Powerscourt 1884	74.6	75.6	68.0	78.0

UK lag	db3	Time spent in England. Estimated from establishment in Woburn Abbey through arrival in US. 23 years.	4 - 11 gen.	Lowe and Gardiner 1975	2.1	1.8	1.0	5.0
UK founders	Nf3	Number of deer that contributed to Founding of UK from Yakushima Island deer	4 ind.	Powerscourt 1884	3.4	3.9	1.0	4.0
Yakushima Founded	t6	Time spent on Yakushima Island after glaciation split, expected to fall between t5 and t7 generations.	78 - 10,000	No account provided	4700.0	4520.0	480.0	9370.0
Yakushima Founders	Nf4	Population size at split during glaciation.	20 - 5,000	No account provided	24.1	23.2	3.8	47.2
Sika split	t7	Time when "ghost" population and Yakushima population are expected to have diverged (Riss-Würm interglacial period: 100,000 ybp)	plus/minus 25% error. 12,500 - 62,500 gen.	Tamate et al. 1998	33500.0	31700.0	14100.0	58600.0
Mutation rate	Âµmic_1				9.04E-04	9.27E-04	7.30E-04	1.00E-03

Table 4 Estimated allelic richness and private alleles from rarefaction analysis in the computer program HP-Rare in samples from wild Delmarva Peninsula sika deer (Assateague and Dorchester) captive Delmarva Peninsula sika deer (Delaware) and from the source population of the wild sika stocks (Yakushima, Japan). Total alleles observed in each population (N samples) from this study are included.

	Assateague (29)	**Dorchester (54)**	**Japan (14)**	**Delaware (12)**
Allelic Richness	1.1	1.22	1.96	2.28
Private alleles	0	0.03	0.54	0.99
Allele count	11	14	25	28

Table 5 Observed (H_O) and/ expected (H_e) heterozygosity rates across all loci in populations of sika deer from the wild on the Delmarva Peninsula (Assateague and Dorchester), Delmarva Peninsula captive sika deer (Delaware) and the the source population of the wild sika stocks (Yakushima, Japan). Locus and population heterozygosity values highlighted are not in Hardy-Weinberg equilibrium (HWE). Highlighted in yellow are values with heterozygosity deficiency, those in red have heterozygosity excess. Departures from HWE may be a result of missing alleles during the scoring process, which were not evenly distributed between samples or loci.

	Assateague (29)	**Dorchester (54)**	**Japan (14)**	**Delaware (12)**
IGF-1	0.00/0.00	0.00/0.02	0.04/0.04	0.00/0.11
BM4107	0.00/0.00	0.00/0.00	0.35/0.29	0.23/0.27
OarFCB304	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00
BM203	0.13/0.12	0.30/0.25	0.19/0.17	0.05/0.05
BM1225	0.00/0.00	0.00/0.00	0.00/0.31	0.00/0.33
OBCAM	0.00/0.00	0.32/0.22	0.20/0.20	0.07/0.06
RT27	0.00/0.00	0.00/0.00	0.04/0.04	0.00/0.08
BM6438	0.00/0.00	0.00/0.00	0.15/0.14	0.10/0.26
OarFCB193	0.00/0.00	0.00/0.00	0.04/0.15	0.30/0.37
GM4006	0.00/0.00	0.00/0.00	0.04/0.04	0.14/0.25

Table 6 Degree of genetic differentiation among populations (F_{ST}) describing genetic structure of samples of sika deer from the wild on the Delmarva Peninsula (Assateague and Dorchester), Delmarva Peninsula captive sika deer (Delaware) and the the source population of the wild sika stocks (Yakushima, Japan) as measured by GeneAlEx. Values below the diagonal are F_{ST}, values above the diagonal are P values from AMOVA from GeneAlEx.

	Assateague	**Dorchester**	**Delaware**	**Japan**
Assateague		0.001	0.001	0.001
Dorchester	0.094		0.001	0.001
Delaware	0.624	0.633		0.001
Japan	0.220	0.255	0.302	

Table 7 F-statistics from all loci. Statistics marked with an * significant (α = 0.05) from AMOVA performed in the program GeneAlEx.

	IGF-1	BM4107	OarFCB304	BM203	BM1225	OBCAM	RT27	BM6438	OarFCB193	GM4006	Total
Fst	0.025	0.674*	N/A (monomorphic)	0.307*	0.721*	0.246*	0.093*	0.796*	0.631*	0.566*	0.544*
Fis	0.682*	-0.052	N/A (monomorphic)	-0.098	0.254*	-0.366	0.653*	0.109	0.329*	0.387*	0.016
Fit	0.690*	0.657*	N/A (monomorphic)	0.239*	0.792*	-0.029	0.685*	0.818*	0.752*	0.734*	0.551*

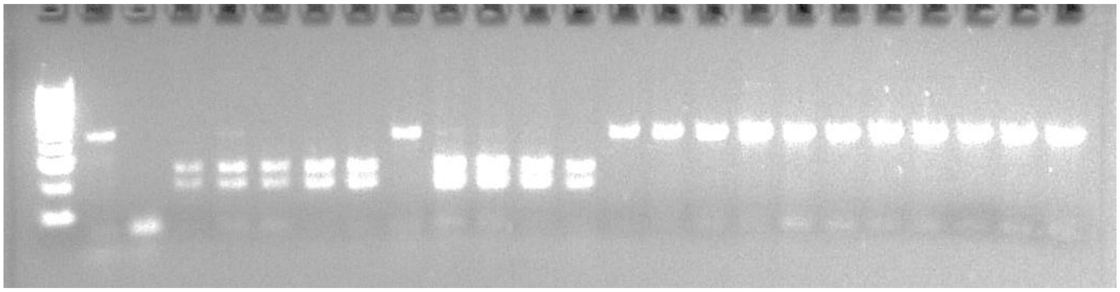

Figure 3 Fragment length polymorphism restriction results to confirm the species of samples that were collected by hunters in Maryland. Lane 1: 1000bp ladder, lane 2: + control sika, lane 3: – control PCR cocktail, lanes 4-8, and 10 – 13 are white-tailed deer which was cut by restriction enzyme HinF1 into two pieces (198+265bp), lane 10 and 14 – 24 are sika deer which were not cut by the enzyme (464bp).

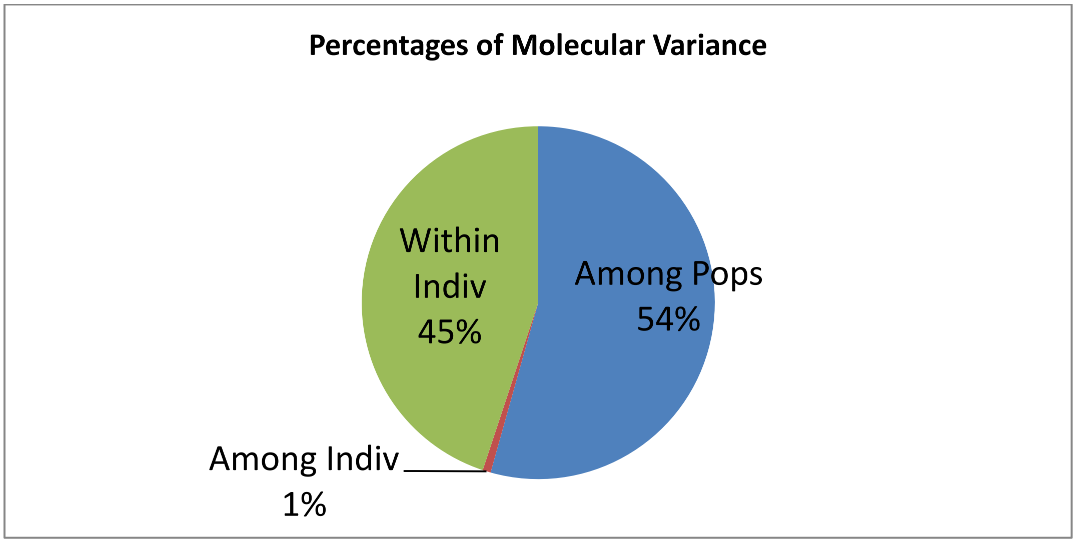

Figure 4 Percentage of genetic variation across sampling areas assigned to among populations, among individuals or within individuals.

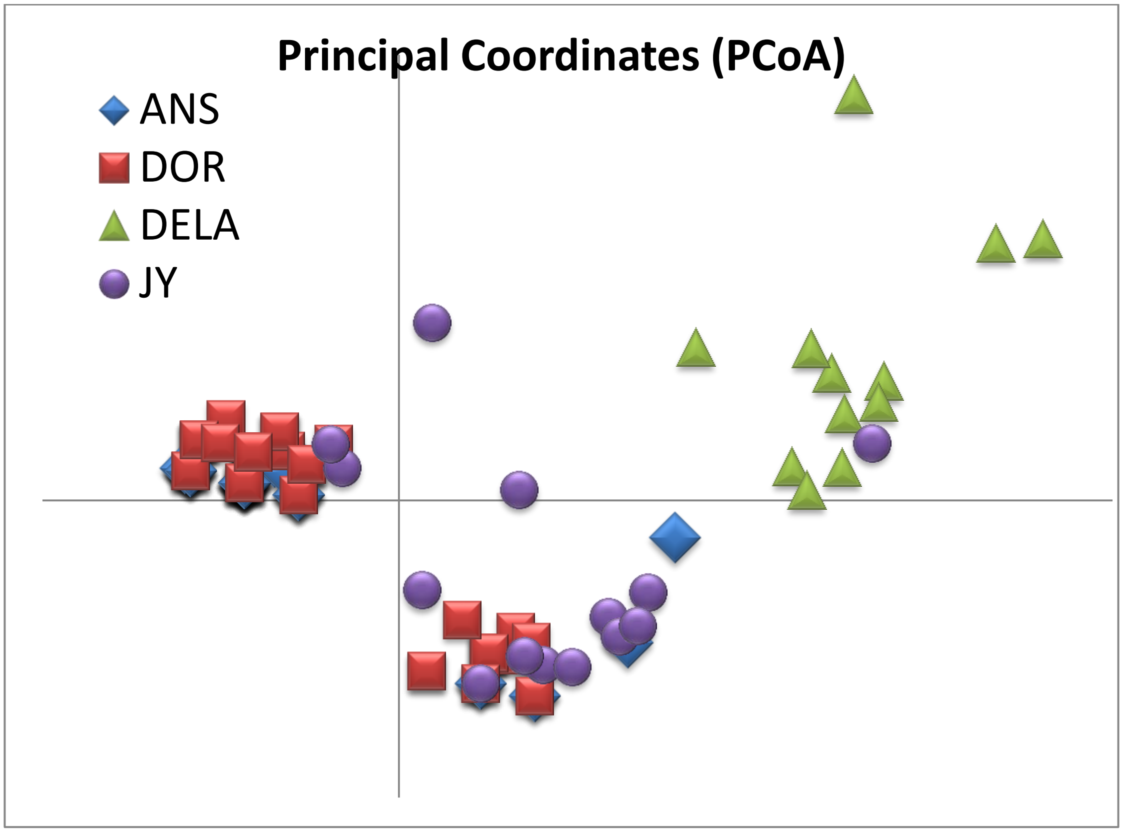

Figure 5 Principle coordinate analysis of percentage of genetic variation across sampling areas of sika deer from the wild on the Delmarva Peninsula (Assateague [ANS] and Dorchester[DOR]), Delmarva Peninsula captive sika deer (Delaware[DELA]) and the the source population of the wild sika stocks (Yakushima, Japan[JY]). Individual assignment to populations was done using 9 polymorphic microsatellites. Across all samples, 44% of the variation in genetic distance was explained with the first coordinate axis, the second axis explained an additional 10% of the variation in our samples, and the third (not shown) an additional 8%.

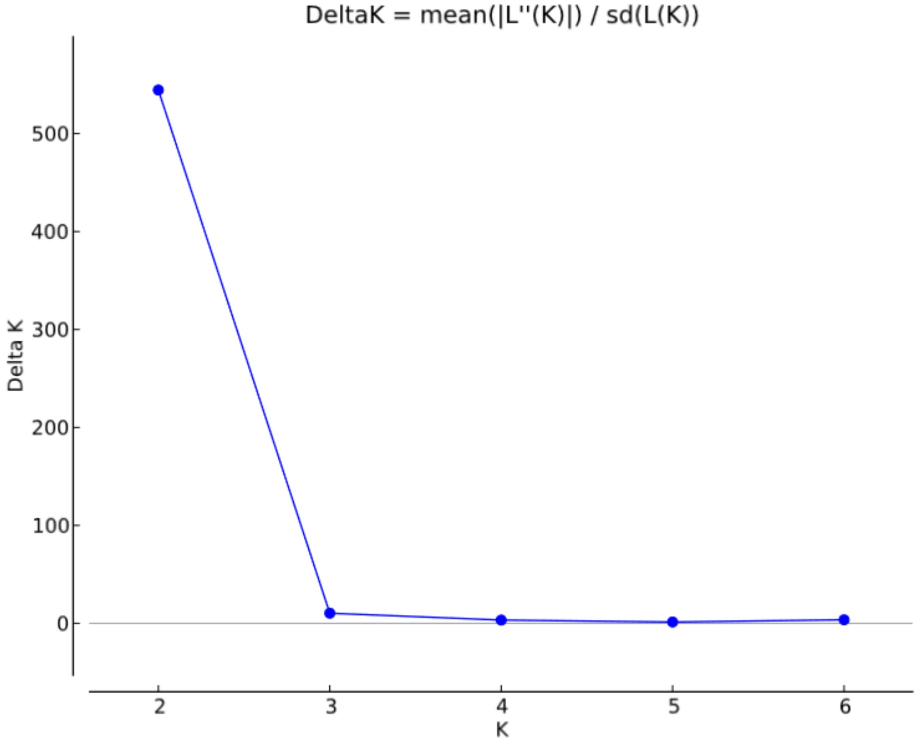

Figure 6 Rate of change in the log probability of sequential K values of wild Delmarva Peninsula sika deer (Assateague, Dorchester), captive Delmarva Peninsula sika deer (Delaware) and the source of wild sika deer (Yakushima Japan) sika deer samples as measured from 10 loci. We ran Bayesian clustering Markov Chain Monte Carlos (MCMC's) with a burn-in of 100,000 each followed by 1,000,000 iterations. We estimated clusters (K) from 1 through 7 (n+3) with 8 iterations of each cluster. Population cluster assignment was estimated according to a slight modification of the Evanno et al. (2005) method in STRUCTURE Harvester (Earl and vonHoldt 2012).

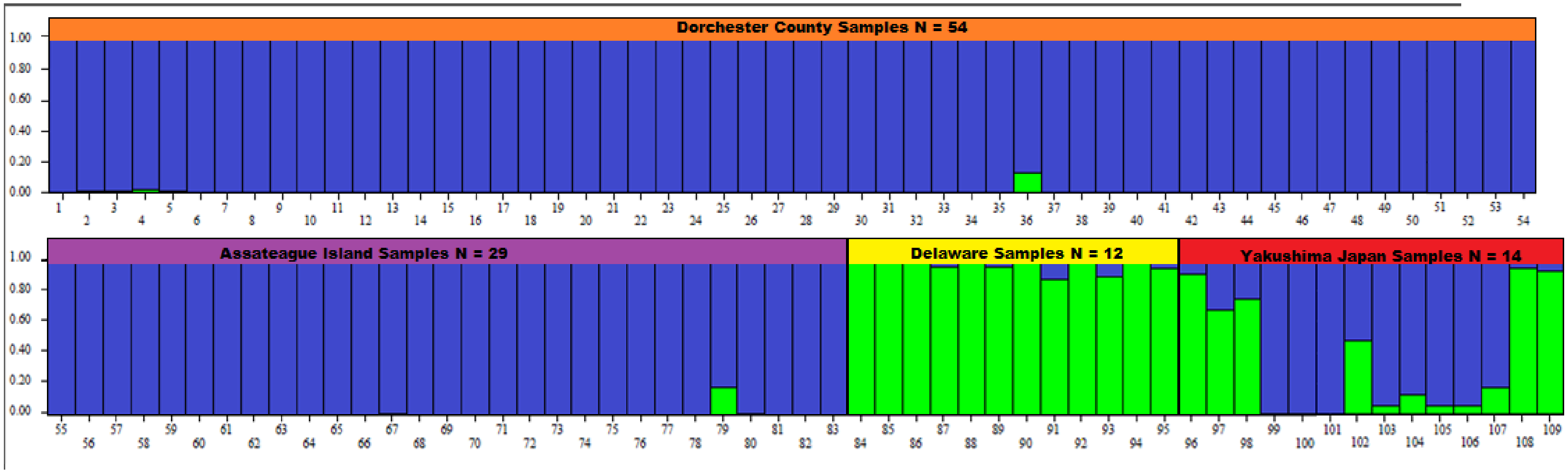

Figure 7 Analysis of Bayesian clustering identified two cluster groups (blue and green) based on 10 loci across all sample populations of sika deer from the wild on the Delmarva Peninsula (Assateague and Dorchester), Delmarva Peninsula captive sika deer (Delaware) and the source population of the wild sika stocks (Yakushima, Japan). Population cluster assignment was estimated according to a slight modification of the Evanno et al. (2005) method in STRUCTURE Harvester (Earl and vonHoldt. 2012). Colored bars above each individual are the sampling location, and the numbers are individual deer samples. Orange bar is Dorchester samples N=54, violet bar is Assateague samples N=29, yellow bar is Delaware samples N=12, and red bar is Yakushima Japan samples N=14.

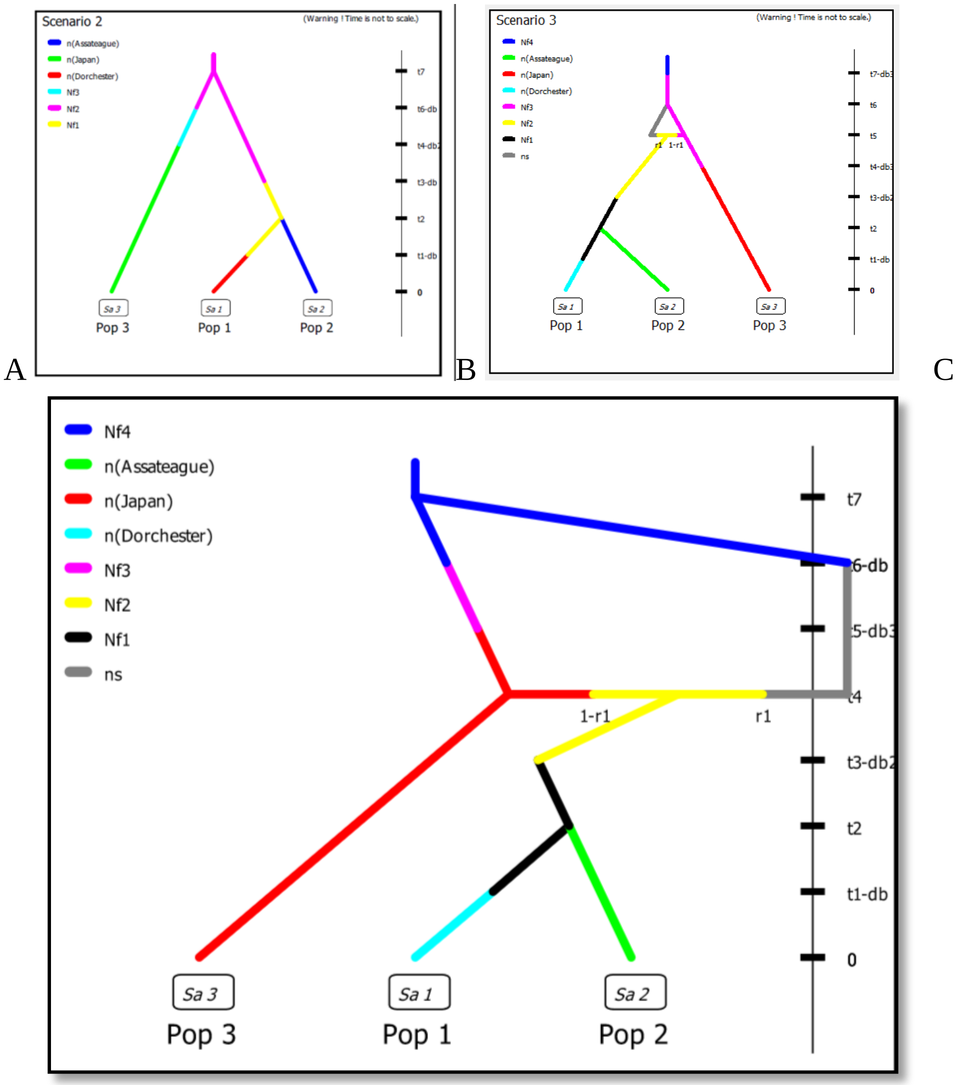

Figure 8 Top historical models selected by DIYABC. Colors are not consistent between lettered images. Timelines on the right are not to scale. Change in colors within images represents a population split, or a population bottleneck. Time 0 represents the current (collection) date. Image C, (enlarged) was selected as the best of all models. The right side from time periods t3 – t6 are equivalent to sika deer being in the United Kingdom, and time periods 0 – t3 represent sika deer on Delmarva (wild deer only).

Chapter 3

DIETARY RESOURCE USE AND COMPETITION BETWEEN WHITE-TAILED DEER AND INTRODUCED SIKA DEER

Abstract

The ecological principle of competition states that resource use among sympatric species evolves to create a fundamental dietary niche. Individuals must adapt their dietary niche to coexist, or competition will ultimately lead to the exclusion of one species. The introduction of exotic species can create competition for resources that may force native species to alter their dietary niche. We investigated the change in niche breadth of white-tailed deer when they share habitat with exotic sika deer. We measured three species interactions that quantify competition between species: change in niche breadth, degree of resource overlap, and a decline in resource quality. During the winter, when resources were most limited, white-tailed deer diet was dominated by woody browse. Sika deer consumed high amounts of both grasses and woody browse during the winter. We observed up to 108% increase in white-tailed deer niche breadth with more than 84% dietary niche overlap with sika deer when they were sympatric. In sympatry with sika deer, white-tailed deer consumed a wider range of lower quality (17% decrease) grasses and forbs. Competition for resources used by white-tailed deer could contribute to their decline over time and allow for the continued spread of sika deer.

Introduction

Natural selection acts to partition sympatric species into unique niches within an ecosystem. This process results in a dynamic balance in nature, where species use resources differently, or use resources at different times (Bell 1971, Putman 1996, Whitney et al. 2011). Sympatric species have coevolved to partition resource use and thus minimize competition (Latham 1999). Exotic species may negatively influence an ecosystem through competition with native species for space and food resources (Kiddie 1962, Baccus et al. 1985, Spear and Chown 2009).

White-tailed deer are the only large mammalian herbivore native to Delaware and the eastern shores of Maryland and Virginia (hereafter, the Delmarva Peninsula). Sika deer were introduced to Delmarva in 1916, and have since increased in number and geographic distribution across the Peninsula. Sika deer and white-tailed deer are similar-sized ungulates that and share similar habitats (Eyler 2001, Kalb et al. 2013), causing concern about their interactions with white-tailed deer (Eyler and Timko 2014). Specific concerns about the interactions of the 2 species revolve around dietary resource use and niche partitioning. While niche involves more than diet, a species' diet drives both the size and shape of its niche (Putman 1996). Dietary overlap between native and introduced species is one of the most pressing areas needed in ungulate research (Demarais et al. 1990, Putman 1996).

Understanding interactions between exotic species and native species are a challenging but essential step in the effects of exotic species on plant and animal communities (Roy et al. 2009, Hesketh et al. 2010). While conceptually straight forward, competition is often difficult to measure quantitatively in field settings (Putman 1996) because it affects species in multidimensional and synergistic ways. For example, shifts in space and resource may obscure competitive interactions. Accordingly, most studies of niche overlap and competition have focused on site-specific interactions in a simple community (Anthony and Smith 1977, Ludewig and

Bowyer 1985, Faas and Weckerly 2010). Furthermore, most studies of niche overlas lack proper controls (Faas and Weckerly 2010).

We quantified resource use, and change in use for both white-tailed deer and sika deer under conditions of sympatry and allopatry throughout the Delmarva Peninsula. The resulting data were used to test the hypotheses that sika and white-tailed deer diets overlap, and that diet composition and quality for white-tailed deer is lower where white-tailed deer are sympatric with sika deer than when the 2 species are allopatric. We summarize these data to quantify competitive interactions between the two species.

Study Area

We selected three study areas on the coastal plain of Maryland based upon the species composition of sika deer and white-tailed deer, similarity of habitats, and similarity of hunting pressure. We selected a study area for each of the following abundances: 100% sika deer (Blackwater National Wildlife Refuge [Blackwater]), 50% of each sika deer and white-tailed deer (Tudor Farms LLC [Tudor]), and 100% white-tailed deer (Eastern Neck National Wildlife Refuge [Eastern Neck]).

Tudor (38.45965,-75.96605: Figures 9) was 2,752 hectares of privately owned property in the western portion of Dorchester County, MD. Based on 4 years of infrared camera surveys (Jacobson et al. 1997), in 2012 the white-tailed deer to sika deer ratio was close to 1:1 (S. Q. Dougherty and J. L. Bowman, University of Delaware unpublished data; A. Jolicoeur, Tudor Farms LLC personal communication). White-tailed deer and sika deer were observed across the entire study area, and within all major habitat types in the study area (Figure 10).

Blackwater (38.4221437,-76.2221194: Figures 9 and 11) was composed of several, dis-contiguous sections totaling 10,900 hectares throughout the eastern and southern portions of Dorchester County. Based on personal observation by the author, local property owners, and refuge staff, several of the sections have been dominated by sika deer for several years. To select a section for this study, we reviewed data collected during spot light surveys between 1989 and 2010 in several areas of Blackwater. From these data, we concluded that area M (1150 hectares) was likely to be inhabited by only sika deer, with no white-tailed deer being observed during any of the surveys (Unpublished data: M. Whitbeck, Wildlife Biologist, Chesapeake Marshlands NWR). To complete the Blackwater study area, we added 6 additional cells on private properties to make a contiguous group of 25 grid cells.

Eastern Neck (39.0386189,-76.2360134: Figures 9 and 12) was a 925 hectare island National Wildlife Refuge located in the southwestern portion of Kent County, MD. To date, no one on the refuge has ever observed a sika deer and none have been harvested (Figure 1). To complete the Eastern Neck study area, we added 8 grid cells to the north of Eastern Neck on private property. These cells were contiguous among themselves, but a channel separated the 17 refuge cells from the 8 additional cells on the mainland.

All study sites had similar weather conditions year round. The 30-year averages (1981-2010) for daily low and high temperatures at Eastern Neck were -3.4ºC and 4.4ºC in January, and 20.2ºC and 30.2ºC in July (Chestertown, MD station; NOAA 2015*a*). Eastern Neck averaged 112 cm of precipitation each year with <42 cm of it deposited as snow (Chestertown, MD station; NOAA 2015*b*). The 30-year averages (1981-2010) for daily low and high temperatures for Dorchester County were

-2.4ºC and 8.1ºC in January, and 20.2ºC and 32.3ºC in July (Vienna, MD station; NOAA 2015*a*). Dorchester County averaged 112 cm of precipitation each year with <14 cm of it deposited as snow (Vienna, MD station; NOAA 2015*b*).

We collected the information regarding deer abundances from hunter harvest data, camera surveys, and spotlight surveys (Beemiller, C. Refuge Manager, Eastern Neck National Wildlife Refuge; Whitbeck, M. Blackwater National Wildlife Refuge System Biologist; Bowman, J. University of Delaware Unpublished data). All sites had a limited number of days of the season that were open to hunters and a limited number of hunters that were allowed access. Harvest information for Dorchester County was collected from the Maryland annual deer report (Eyler and Timko 2014).

Methods

Study site selection

We compared several potential study area habitats from Land Use Land Cover (LULC: USGS 2011) data to find areas that provided similar spatial coverages of habitat types. We then ground-truthed the LULC habitat types to verify the vegetation in the habitats were similar across study areas. We identified vegetation in 6-8 random cells from each study area, with at least one from each LULC type: upland forest (mixed deciduous or coniferous), woody wetland, open marshland and agricultural. We used the starting point of centralized transects within random cells as the point reference for vegetation sampling during fecal collections. We followed the vegetation sampling technique in the U.S. Army Corps of Engineers Wetland Delineation Manual (2008) for fast and accurate identification of the vegetation community. We identified all plant species within the 4 strata layers (trees, shrubs, herbs, and woody vines) to the nearest 5% of the plot's composition.

Transects and fecal collection

We divided each study area into 25- 800m by 800m (64 ha) cells. Individual cell size was based on the 95% kernel female white-tailed deer home range (Eyler 2001, Rhoads et al. 2010). Whenever possible, we placed cells systematically across the study areas to create a grid, but attempted to avoid large areas of open water (Figures 10, 11, and 12).

We established a sampling transect within each cell starting as close to the center of the cell as possible. To increase our collection efficiency we used deer trails as transects (Brinkman et al. 2011). We selected the start of each transect by locating the deer trail closest to the center of the cell that was not under water and that did not cross onto property where we did not have permission to access. We marked each transect using a handheld global positioning system (GPS) unit, and forestry flagging so that exact transects could be revisited. We walked each transect for 200 m from the start and collected all suitable fecal samples that we encountered (Jenkins and Manly 2008). We followed a random bearing along each transect so if another deer trail intersected our sampling transect, we selected the trail that traveled closest to the original bearing (Brinkman et al. 2011). We measured the distance from the starting point to each fecal group collected, and removed all unused fecal pellets from transects.

We collected samples during winter (December – February), the non-growing season, when forage resources were the most limited (Taillon et al. 2012; Seto et al.

2015). We sampled each transect twice, about 7 days apart (range 4 – 14 days at Tudor, exactly 7 days for Blackwater and Eastern Neck: Brinkman et al. 2010, Brinkman et al. 2011) after the first sampling date. We sampled transects with two observers on each run, or twice by the same observer (forward and backward) per run to ensure that we did not miss any pellet groups (Jenkins and Manly 2008).

We collected samples at Tudor in 2011, at Blackwater in 2012, and at Eastern Neck in 2013. In 2013, we collected additional samples from Tudor and Blackwater to compare diets between years. We collected these additional samples from random transects within the same grid cells but from different deer trails.

We visually estimated the quality of all fecal samples based on the pellet color, consistency, and sheen (Brinkman et al. 2010). Quality rankings ranged from a 3 (highest quality, fresh, sticky with mucus, a slight sheen on pellets that were soft and/or warm) to 1 (lowest quality, firm and dry but still had a sheen of mucus). We did not collect or count pellet groups that showed signs of mold, or that appeared broken through decay, or lacked a sheen of mucus on the exterior.

We placed all samples into sterile 50-ml centrifuge tubes containing 25 ml of 95% ethanol (enough to cover the sample); we changed gloves between samples (Brinkman et al. 2010). For all samples, we recorded a unique collection number, collection date, transect number, distance from the start, number of pellets collected, and quality of the pellet. We attempted to differentiate species based on fecal pellet size but were unsuccessful (APPENDIX A). We stored all samples at room temperature in a dark cabinet until DNA extraction.

Fecal DNA identification

We extracted DNA from fecal pellets following Wehausen et al. (2004). We used either Qiagen (Venlo, Limburg, Netherlands) or Bioline's (London, UK) extraction kit for fecal samples. We stored extracted DNA at -80°C.

For each sample, we performed a restriction fragment length polymorphism (RFLP) assay to identify the species. We used sets of known tissue samples from across the range of our study areas and identified a set of primers that would amplify a section of the mitochondrial D-loop resulting in a fragment of ~464bp in length for sika deer (Wolf et al. 1999) and white-tailed deer. We identified differences in base-pair sequences from the National Center for Biotechnology Information (NCBI: GenBank) for both sika deer (Wada et al. 2007) and white-tailed deer (Seabury et al. 2011). We modified the light strand primer from Wolf et al. (1999), L14735, at 2 separate base pair point changes: transitions at position 9 C to T, and position 16 T to C so that our primers better fit both deer genomes (sika deer: Wada et al. 2007; white-tailed deer: Seabury et al. 2011).

5': AAA AAC CAT CGT TGT CAT TCA ACT A

The heavy strand primer (H15149) differed in two positions for sika deer and one position for white-tailed deer (Wada et al. 2007, Seabury et al. 2011), all at unique locations, and therefore we did not modify it. We amplified this fragment using the polymerase chain reaction (PCR), with an initial 3-min denaturation at 96 °C, followed by 45 cycles (96 °C for 30 s, 60 °C for 1 min) and final extension at 72 °C for 3 h (Wolf et al. 1999). The reaction mix included 5µg of each primer (Invitrogen; Carlsbad, CA), 0.05µg BSA (Promega;Madison, WI), 12.5µL MyTaq Red Mix (Bioline), about 50 ng DNA template, and enough DI water to bring the final volume up to 25 µL.

Based on the sequence comparisons (Wada et al. 2007, Seabury et al. 2011), we selected restriction enzyme HinF1, which cut fragments that were large enough to be viewed easily on an agarose gel. HinF1 cut G│AN(R=[A])TC in white-tailed deer, resulting in two fragments (198bp and 265bp), but did not cut the sika deer fragment (Figure 3). The restriction cocktail consisted of 5 units of HinF1, 0.025µg BSA (Promega), 2µL RE 10X Buffer (Promega), 5 µL of the PCR products, and was brought to a final volume of 10 µL with DI water. We digested the samples at 37°C for 3 hours, then we electrophoresed the products on a 2.0% agarose gel containing ethidium bromide (5µL per 100 µL of agarose mix) and visualized results under UV light. . We amplified and restricted all samples with known positive and negative controls, and ≥2 viewers independently confirmed the results.

General diet analysis

Dietary comparisons can be successfully done through microhistological analysis, which compares undigested food particles found within a fecal sample to known plant samples (McCullough 1980, Wegge et al. 2006, Bertolino et al. 2008, Kufner et al. 2008). We sent samples for microhistological analysis to the Washington State University Wildlife Habitat Nutrition Laboratory, Pullman, Washington (Colligan et al. 2011, Whitney et al. 2011). Once we identified the species that defecated the fecal pellets, we grouped samples to investigate diet.

We created 18 composite diet samples from 278 identified fecal sample groups (Table 8). We combined fecal pellet samples into composite dietary groups because our interests were in general dietary resource overlap between species (i.e. what proportions of diet were shared resources) rather than individual resource use, additionally composite diet groups were more fiscally conservative. We grouped our samples by species, study area, and year of collection. From these groups we randomly selected 15 fecal samples to combine into a single composite diet sample. We continued to randomly select fecal samples in sets of 15 until there were not enough samples from a group to make a diet sample. We took 2 fecal pellets from each fecal sample in each diet sample group and combined them to create an overall composite diet sample (Wegge et al. 2006, Bertolino et al. 2008, Kobayashi and Takatsuki 2012, Jung et al. 2015). For species area/year combinations that did not have 15 fecal samples, we took 2 pellets from all fecal samples to create the composite diet sample (Table 8).

We dried all pellets for each diet sample at 50°C for 48 hours before being sent to Washington State Nutrition lab. The lab thoroughly homogenized each diet sample and made 6 slides from each of the 18 composite diet samples. Each slide was viewed 25 times for 150 total views per sample to obtain a 90% confidence interval around 90% of the sample means (Holechek and Vavra 1981, Bertolino et al. 2008, Whitney et al. 2011). Kochenberger (1982) observed that 95% of the plant species observed in sika deer diet were observed within 50 views, so additional views increased our confidence in observing the majority of plant species within any individual diet sample. Where possible the genera of each dietary resource was identified and provided with the percent composition within each diet group. When the species could be identified this was also provided. All diet results were presented in 6 dietary resource groups (grass, forbs, sedges/rushes, woody browse, conifer, and other). Resources in group other included flowers, seeds, nuts, thorns, moss and ferns. We did not apply dietary correction factors because sika deer and white-tailed deer are similar sized cervids and it is unlikely that plant digestibility differs greatly between them (Kobayashi and Takatsuki 2012).

Niche breath

The simplest measure of dietary niche breadth is a count of total number of species consumed, or total numbers of species consumed over any given threshold (Krebs 1999). We evaluated differences in percent consumptive use between white-tailed deer alone to white-tailed deer when with sika deer, sika deer alone to when they were sympatric with white-tailed deer, and both deer species in the same study site, by 5 general resource categories (grass, sedges and rushes, forbs, woody browse, and conifer) with a 2 way Student's T-test of the Arcsin square-root transformed percentages of resource use in Microsoft Excel (group "other" was excluded due to extremely low usage of unrelated items) based on sample variances. These comparisons were determined *a priori*, and significance was set at $P=0.05$.

We also calculated niche breadth with two common measures found throughout the literature. The Shannon-Wiener Information Measure (Colwell and Futuyma 1971, Hanski 1978, Krebs 1999, Kobayashi and Takatsuki 2012) where SB_j is the niche breadth of species j:

$$SB_j = -\sum_j P_{ij} * ln(P_{ij})$$

And Levins' Measure of Niche Breadth (Levins 1968, Hurlbert 1978, Bertolino et al. 2008) where LB_j is the niche breadth of species j and P is the proportion of the i^{th} resources used:

$$LB_j = \frac{1}{\sum P_i^2}$$

Since both measures can range from 0 – ∞, we standardized them to a 0 – 1 scale. The standardized Shannon-Wiener Evenness Measure was calculated by dividing the Shannon-Wiener Information Measure by the logarithm of the n total resources. Standardized Levins' Measure of Niche Breadth was calculated by:

$$LB_j^* = \frac{LB_j - 1}{N - 1}$$

Both indices are important since we are ultimately interested in how the niche of the white-tailed deer changes in the presence of sika deer. We selected these 2 measures for the opposing focus of either measure, the Shannon-Wiener Information Measure gives weight to all resources/resources that are less common in the diets, while the Levins' Measure gives more weight to the resources that are most common in the diets (Hurlbert 1978, Krebs 1999).

Dietary overlap

We measured overlap in 4 different ways. These indices have been modified in presentation so that for each P_{ij} is the proportion of ith resource that is consumed by species j, and P_{ik} is the proportion of the ith resource consumed by species k. We measured the differences in diet groups with Horn's Index of Similarity (Horn's) between diet compositions(Horn 1966, Jenkins and Wright 1988).

$$R_{\circ} = \frac{\sum\left(\mathrm{P}_{ij} + P_{ik}\right)\ \log\left(P_{ij} + P_{ik}\right) - \sum \mathrm{P}_{ij} \log \mathrm{P}_{ij} - \sum P_{ik} \log P_{ik}}{2\log(2)}$$

We measured the degree of dietary niche overlap for diet groups using Pianka's Index of Overlap (Pianka's: Pianka 1973).

$$\propto_{j,k} = \frac{\sum P_{i,j} P_{i,k}}{\left[\left(\sum_{P_{i,j}} 2\right)\left(\sum_{P_{i,k}} 2\right)\right]^{1/2}}$$

We also used the simplified Morisita Index (Morisita's) from Horn (1966) to evaluate overlap (García-Godos et al. 2004).

$$C_H = \frac{2\sum_i^n P_{ij}\, P_{ik}}{\sum_i^n P_{ij}^2 + \sum_i^n P_{ik}^2}$$

We also measured dietary overlap with Schoener's Index of Overlap (Schoener's) due to its strength and wide use (Schoener 1968, Abrams 1980, Wallace 1981, Wolda 1981, Yang and Livingston 1986, Mysterud 2000, Bertolino et al. 2008).

$$C_{jk} = 1 - 0.5 * \left(\sum \left|P_{ij} - P_{ik}\right|\right)$$

These indices of overlap vary from 0 when there is complete niche separation between species, and 1 when there is complete overlap between species (Horn 1966, Schoener 1968, Pianka 1973, Putman 1996). Values for these indices of overlap are generally considered significant when they are greater than 0.60 (Zaret and Rand 1971, Mathur 1977, Wallace 1981, Langton 1982). Diet samples from the same species/area/year were grouped for overall analysis (Tables 8). Diets from the same area/species were grouped acrossed years when there was significant overlap (>0.6) in all 4 indices of overlap.

Diet quality

We estimated the quality of forage items within the diets of both deer species based on their nutrition and palatability values from previous literature (USDA plants database, USFS database). When possible we selected values based on winter months. From the USDA grading system, we gave each dietary resource a grade (1; poor, 2; moderate, 3; fair, 4; good, and 5; very good). When we observed a resource in the diet without a nutritional value, we averaged the closest related species (locally found within the genera) to create a grade (Table 9). When separate information regarding quality of the resources were provided, we assigned a value for white-tailed deer from white-tailed deer nutritional values, and assigned sika deer values based on elk (*C. elaphus*) which are the closest related species common to North America and are expected to be able to digest resources similarly as intermediate browsers (Hofman 1989). When only one nutritional quality was provided for deer or ungulates, we assigned that value to both sika deer and white-tailed deer. We assigned unknown samples within our observed resources an average value based on all the known resources within each major dietary category (i.e. grass, forb, woody browse). Based on browsing characteristics and nutrient qualities, we assigned stem and twigs a lower value than the forage values for leaf in all species that had both a leaf and twig component observed in the diet.

To determine the changes in the quality of deer resource use, we multiplied the proportion of each resource used by the assigned resource quality, and summed all these values for each composite dietary sample to create a total diet quality index. We determined percent change in resource quality by averaging the composite dietary sample resource qualities by species within study areas. We used a Student's T-test to measure differences in quality index between pairs of deer diet groups. We used a Bonferroni correction factor (Bonferroni 1936, Aickin Gensler 1996) to adjust our P-value for familywise error rates in diet quality comparisons.

Results

Study area selection

In all three study areas, we observed the dominant species in the tree stratum to be loblolly pine (*Pinus taeda*), white oak (*Quercus alba*), northern red oak (*Quercus* *rubra*), willow oak (*Quercus phellos*), red maple (*Acer rubrum*), sweet gum (*Liquidambar styraciflua*), black gum (*Nyssa sylvatica*) and American holly (*Ilex opaca*). We observed the dominant shrub species to be highbush blueberry (*Vaccinium corymbosum*), marsh mallow (*Althaea officinalis*), wax myrtle (*Myrica cerifera*), and tree stratum saplings. We observed the dominant herb layer plants to be common reed (*Phragmites australis*), woodoats (*Chasmanthium* spp.), rush (*Scirpus spp.*), sedges (*Cyperus* spp. and *Carex* spp.), cord grass (*Spartina spp.*), Japanese stilt grass (*Microstegium vimineum*), clover (*Trifolium* spp.), multiflora rose (*Rosa multiflora*), common nettle (*Urtica dioica*), spikerush (*Eleocharis parvula*), and cattail (*Typha* spp.) with a shift in plant dominance up tidal gradients (Feldhamer and Demarais 2009). We observed the dominant woody vine plants to be poison ivy (*Toxicodendron radicans*), and greenbriar *(Smilax* spp.). All agricultural fields were monocultures of corn (*Zea mays*), soybean (*Glycine max*), and/or winter wheat (*Triticum aestivum*).

Few dominant species were observed that were not found at all three sites. Hickory (*Carya spp.*) and black cherry (*Prunus serotina*) were observed in the tree layer at Eastern Neck. Mile-a-minute (*Persicaria perfoliata*) was also observed at Eastern Neck. American beech (*Fagus grandifolia*) was observed in the tree layer at Tudor. We did not observe any dominant plants at Blackwater that were not also found at the other two sites. We did not completely survey each cell because our interests were in creating an index of dietary overlap rather than specific details of resource use for either species. Given their regional proximity (all study sites were within 100 km of each other) and dominant plant composition within cover types, we are confident that the minor differences in plants between sites would not bias our resource use measurements.

Fecal samples

We collected 1328 fecal samples (Table 10). We were able to identify the species of 278 of these samples via molecular analysis (Table 10). We used 260 of the speciated samples in 18 diet groups to determine deitary use and overlap (Table 8). We collected 145 samples at two sites (Tudor and Blackwater) a second time and our success rate greatly improved at both study areas in the second year of collection (Table 10).

Dietary use

When they were sympatric, sika deer consumed more grasses ($T_9 = 2.40$, $P = 0.040$) and more sedges/rushes ($T_9 = 4.40$, $P = 0.002$) than white-tailed deer and white-tailed deer consumed more woody browse ($T_7 = 4.57$, $P = 0.003$) and more forbs ($T_9 = 2.28$, $P = 0.048$) than sika deer (Table 11). Sika deer alone did not differ in percent consumption of any resource category compared to when they were sympatric with white-tailed deer (Table 11). White-tailed deer alone consumed more conifers ($T_9 = 2.63$, $P = 0.027$) compared to when they were sympatric with sika deer, while white-tailed deer sympatric with sika deer consumed more sedges/rushes ($T_9 = 3.40$, $P = 0.014$) than white-tailed deer alone (Table 11). White-tailed deer sympatric with sika deer trended towards consuming more forbs ($T_9 = 2.12$, $P = 0.063$) than white-tailed deer alone.

All of our observed deer diets were comprised of 54 different resources (Table 10). Of these, 8 were unknown (an unknown grass, forb, shrub, conifer, fern, lichen, nut, or thorn). Of the remaining 46 resources, 7 included both a leaf and stem component; all but 16 of these 46 resources were identified to species. Five resources were consumed by both species in all three study areas *Quercus* spp., *Myrica* spp., *Pinus* spp., *Fescuta pratensis* and *Hordeum pusillum.*

Excluding unknowns, there were 7 resources found only at 1 study area. Each of these 7 resources comprised less than 4% of the total diet of the sample they were found in. We observed 4 of these 7 only in white-tailed deer diet when they were with sika deer. These 4 resources were all forbs (*Bidens spp., Chamaecrista fesciculata, lepidium virginicum*, and legumes). White-tailed deer at Eastern Neck (when alone) consumed *Amelanchier canadensis* which was not found in other study area diets. The other 2 resources were found at Tudor, and were consumed by both sika deer and white-tailed deer (*Elaeagnus spp.* and *Melilotus spp.*).

Ten known resources observed in white-tailed deer winter diet were not found in sika deer diet (6 browse plants: *Rubus* spp., *Rhus copallina, Lonicera* spp., *Carya* spp., *Betula niger, Amelanchier canadensis* and 4 forbs: *Lepidium virginicum, Bidens* spp., and *Chamaecrista fasciculata*, and legumes [Table 9]). One known resource found in sika diet was not observed in white-tailed diet (a grass: *Setaria* spp. [Table 9]).

Niche Breadth

We observed a 44% increase in the resource diversity when white-tailed deer were with sika deer compared to when they were the only ungulate present (average 26.4 resources vs. 18.4 resources). When white-tailed deer shared habitat with sika deer, they increaed their use of 5 grass species, 4 sedge/rush species, 8 forbs, 12 woody browse species, and ferns, lichen, and nuts, compared to use in habitat without sika deer. Eighteen resources used by white-tailed deer with sika deer were not observed in samples from white-tailed deer when they were alone. White-tailed deer alone consumed 3 resources that we did not observe in white-tailed deer when with sika deer (1 grass: *Holcus lanatus,* 1 woody browse: *Amelanchier canadensis,* and thorns).

We observed some change in sika deer resource use, but not to the extent we observed in white-tailed deer. Sika deer increased resource use by 14% when they were with white-tailed deer. Sika deer used an average of 19.5 resources while they were alone compared to an average of 22.2 when they were with white-tailed deer. White-tailed deer always had a smaller niche breadth (4 – 77% smaller than sika deer) from their comparative sample area (alone or with the other species) across all measures of niche breadth (Table 12). The widest niche breadths for both sika deer and white-tailed deer occurred when both species were present (Table 12). The differences in niche breadth size increase from when deer were alone to when they were with the other species were more extreme for white-tailed deer, showing an increase in niche breadth between 18% and 108%. Sika deer niche breadth increase fell between 2% and 42% (Table 12). Niche breadth of both white-tailed deer and sika deer samples collected at Tudor in the second year of collection were similar to the niche breadths of each species.

Indices of overlap

Schoener’s Index (subscript S) often resulted in our most conservative estimates of dietary overlap (and most often regarded as the best: Linton et al. 1981, Wallace 1981, Wolda 1981) while Horn’s Index (subscript H) was generally the most liberal with Pianka’s (subscript P), and Morisita’s (subscript M) falling inbetween (Tables 13, and 14). Diets for Tudor and Blackwater between years had a high degree of overlap with samples from the same species ($0.68_S – 0.94_P$) and were pooled with the previous year's samples (we did not sample a second year at Eastern Neck).

We observed a high degree of overlap between sika deer diets when they were alone and when they were with white-tailed deer ($63_H – 88_P$ %: Tables 13, and 14). We also observed a high degree of overlap between white-tailed deer diet samples when they were alone and when they were with sika deer ($45_S – 76_H$ %: Tables 13, and 14). Indices of overlap were also high between sika deer and white-tailed deer when they shared habitat ranging from $55_S – 85_H$% (Tables 13, and 14).

Resource quality

White-tailed deer (alone and when with sika deer) maintained a higher dietary quality (7% - 85% greater) than sika deer both alone and with white-tailed deer (Table 15). White-tailed deer forage quality was significantly ($T_7 = 4.128$, $P = 0.004$) lower when they were with sika deer (range 204 – 284) compared to when they were the only ungulate (range 270 – 288; Table 14, Table 15). White-tailed deer diet in the presence of sika deer was 17.2% less nutritious (Figure 14, Table 15). Sika deer dietary nutrition estimates changed less than 1.1% when they were alone (range 159 – 193) compared to when they were with white-tailed deer (range 165 – 191) despite there being changes in the resources that they used (Figure 13).

Discussion

The diet of any species has implications on the activity and success of that species. In an environment where white-tailed deer diet is affected by the presence of an exotic ungulate that consumes the same resources, we expect to find changes in one or both species compared to when the deer are alone. While we observed changes in both species dietary uses, those observed in white-tailed deer were more dramatic, and had a negative effect on white-tailed deer.

Fundamental dietary niche involves the theorized total consumptive use of a species. We expected the diet of white-tailed deer from Eastern Neck to exhibit a natural fundamental dietary niche for the species. When fundamental niche increases, or when the diet changes outside of this fundamental niche we can attribute the change to competition (Schoener 1974, Putman 1996). In all measurements of niche breadth, we observed small changes in sika deer. White-tailed deer increased niche breadth from when they existed alone to when they coexisted with sika deer. We attribute the increase in resources used to the exclusion of white-tailed deer from their preferred resources. Our findings support others who have suggested that sika deer have a high potential for competition with white-tailed deer when resources are limited (Keiper 1985, Jackley 1991).

We observed a similar or greater degree of overlap between white-tailed deer and sika deer (56 – 84%) than has been observed with native ungulates. Sika deer and white-tailed deer on the Delmarva Peninsula are not spatially or temporally segregated from the same habitat use, which compounds the implications of resource overlaps. In areas where white-tailed deer and other native deer co-occur, dietary overlap is lower than what we observed (41.2% with moose [*Alces alces*]: Ludewig and Bowyer 1985). When in the same habitat as black-tailed deer (*Odocoileus hemionus*), diet was found to have a high degree of overlap, but space use was substantially reduced (highest overlap was 40%) and the two species were found to mutually avoid one another. Jenkins and Wright (1988) compared the dietary overlap of both moose and elk (*Cervus elaphus*) with white-tailed deer which ranged from 25 – 57% for moose, and

68 – 81% for elk. The high degree of overlap with elk was partially attributed to extreme weather conditions where both species were restricted in resource diversity and availability. Anthony and Smith (1977) also compared dietary overlap of mule deer with white-tailed deer. The overlap with mule deer was measured with Schoener's Index of Overlap and varied from 55 – 67% between seasons with the greatest overlap observed in the fall, but again there was a high degree of spatial separation (Anthony and Smith 1977).

The coevolution of white-tailed deer with elk, mule deer, black-tailed deer, and moose have allowed these species to partition their niches in different ways which allow higher degree of overlap in diet, yet reduced competition. White-tailed deer avoid overlap with other natives by geographical separation (often elevation) within the same area (Anthony and Smith 1977, Krausman and Ables 1981, Jenkins and Wright 1988, Whitney et al. 2011). White-tailed deer and sika deer have only begun to share habitat, so resource use is not partitioned between them.

The resources that overlapped between sika deer and white-tailed deer were important resources in white-tailed deer diet. While only 45% of the resources used by white-tailed deer when alone were consumed by sika deer, those resources comprised almost 80% of white-tailed deer diet. When sharing habitat with sika deer, white-tailed deer were forced onto a wider variety of resources. Sika deer also increased the number of plants consumed when sharing habitat with white-tailed deer, but to a lesser degree (15%). The low degree of change in sika deer diet shows that they are not affected by the presence of white-tailed deer and able to acquire the resources they need. Both species increased resources used when sympatric; the effects of this are apparent in the degree of overlap that exists between their resources used.

Winter is a nutritionally critical period for ungulates, especially when in cold weather climates (Taillon et al. 2012, Seto et al. 2015). The winter is when we expect to find resources most limited, and deer selecting a wider range of plant resources to obtain the required nutrients. White-tailed deer are known browsers, and they become stressed when browse diet is limited or reduced and therefore are forced to rely more on grasses and other lower quality resources (Baccus et al. 1985, Demarais and Osborn 1988, Jackley 1991). Sika deer are known generalist grazers, with seasonal diet preferences that fit both browsing and grazing categories and they are extremely dynamic in their resource use in many different habitat types (Kochenberger 1982, Takatsuki 1988, Putman 1996, Kobayashi and Takatsuki 2012, Seto et al. 2015). Our data also show a high degree of woody browse use by sika deer in the winter which is where dietary overlap is accentuated.

The use of fecal samples for dietary resource use was valuable in creating a competition index. Fecal pellets identification followed previous composition estimates on all 3 study areas allowing us to compare resource use with and without both species present. The improvement in DNA amplification from the first year to the next two is likely a result of differences in scoring of fecal quality. The constant inundation and precipitation, made samples wet and soft creating the appearance of a higher quality sample that were initially difficult to distinguish from true high quality samples.

Our study design allowed us to measure competition across areas of similar resource availability. Only 7 of the 46 total resources were not observed across study areas, and 4 of these were only observed in white-tailed deer when they were with sika deer where they competed for resources. These unique resources may be last resort food sources to fill the stomach of white-tailed deer despite toxic or low quality attributes (Laycock 1978).

The evolutionary history of sika deer allows them to take advantage of highly available (lower quality) resources. When we estimated the quality of diets of both white-tailed deer and sika deer, we observed a decrease in the nutritional value of white-tailed deer diet when they are with sika deer. Sika deer diet quality was not affected by the presence of white-tailed deer. The decline in dietary quality in white-tailed deer, combined with the high degree of overlap, and increasing niche breadth of white-tailed deer when with sika deer provide a clear picture of resource competition.

Decreased nutrition can cause a decrease in growth and body size, but also affects fecundity, lactation and juvenile survival, which causes decreased recruitment and a decline in the abundance of white-tailed deer (Verme 1969, Langenau and Lerg 1976, Taillon et al. 2012). As little as 20–30% decrease in nutritional quality can result in dramatic effects on reproductive success and survival (Verme 1969, Langenau and Lerg 1976). Our observed 17% decline in winter nutritional quality may cause a decline in reproductive success annually, but can be dramatically compounded over years of interactions between these species. In addition, the effects of decreased nutritional quality will be amplified in white-tailed deer in years of scant quality resources (Putman 1996).

On the Delmarva Peninsula, the diet of sika deer has been investigated several times via different techniques (Kochenberger 1982, Keiper 1985, Sturm 2008); however, all of these studies were conducted on the barrier islands of Assateague and

Chincoteague, MD where the habitat is somewhat different than the rest of the Delmarva Peninsula lacking agricultural areas and upland forested areas. In addition to different habitats, the presence of wild ponies complicates the resource use of white-tailed deer and sika deer on the barrier islands. Despite the differences, all of these studies, as well as others, suggest the potential for competition between sika deer and white-tailed deer (Flyger and Warren 1959, Feldhamer et al. 1978, Harmel 1980, Baccus et al. 1985, Davidson and Crow 1983, Keiper 1985, Henke et al. 1988, Keiper 1990, Jackley 1991, Harmel 1992, Feldhamer and Demarais 2009). In Texas, Jackley (1991) found that there may be high competition for important resources during stressed periods (winter). Henke et al. (1988) also observed a large portion of sika deer diet to overlap the predominant white-tailed deer forage. The conclusions of all of these projects are the same; unmanaged populations of sika deer are expected to not only compete, but outcompete white-tailed deer.

A decrease in diet quality as a result of competition could also partially explain the increasing proportion of sika deer in Dorchester County's annual deer harvest (Figure 15) including a 10 year low in white-tailed deer harvested in 2015. We recommend that Maryland open legal harvest of sika deer up to all counties of the Eastern Shore (addition of Cecil, Kent, Queen Anne's), to encourage hunters who see sika deer in these areas to harvest them. We also encourage Chincoteague National Wildlife Refuge to continue pursuing efforts to remove sika deer from the Refuge. Chincoteague NWR currently manages sika deer as a game species and has attempted to create Environmental Impact Statements with the goal of eradicating sika deer (USFWS 2014*b*).

Future studies regarding the interactions of sika deer and white-tailed deer should include an evaluation of the physical interactions (aggression) between sika deer and white-tailed deer. A thorough investigation of the degree of dietary overlap across the entire year (at least monthly) would be helpful in determining when the greatest potential for competition exists (Zaret and Rand 1971).

Acknowledgements

We would like to thank the University of Delaware Department of Entomology and Wildlife Ecology for their financial support to the authors while working on this manuscript. We thank Tudor Farms LLC & P. Jones (including A. Jolicoeur and K. Compton); biologists at Eastern Neck National Wildlife Refuge; biologists at Blackwater National Wildlife Refuge and the many private landowners and managers who allowed access to their properties at many different times of the year M. Aiello, H. Hill, P. Hobbs (including A. Loid), C. Kahl, P. Ransome (including M. Willey), J. Ridgley, R Riggins, T. Strong, M. Strong, and D. Twigg. I thank Dr. B. Vasilas from the University of Delaware, for the time investment to provide expertise on field site hydrology, soils, and plant communities. We also thank the help in the laboratory during several semesters (T. Bonner, H. Kline, L. Newton, M. Kalb, K. Lewis, L. Caprini, and C. Blakely).

Table 8 Composite dietary samples by study area, and species of fecal sample. Groups were made of individual fecal pellet groups drawn randomly from across study areas within a year by species. Up to 15 pellet groups were used to make a diet sample, but for the area/years that we did not have enough of a given species, all samples were used.

	Tudor Farms LLC		**Blackwater NWR**		**Eastern Neck NWR**	
Species	**WTD**	**Sika**	**WTD**	**Sika**	**WTD**	**Sika**
2011 - 2012	6	3	NA	NA	NA	NA
2012 - 2013	NA	NA	-	2	NA	NA
2013 – 2014	1	1	-	1	4	-

Table 9 Proportion of collected fecal samples (from N collected samples) that yielded good DNA based on field estimated fecal pellet quality from each study area. Quality DNA yield improved with sample quality and year collected.

		2011 - 2012		**2012 – 2013**		**2013 - 2014**		
	Quality	**% good**	**N**	**% good**	**N**	**% good**	**N**	
	1	0.11	486			0.51	53	
Tudor	**2**	0.24	268			0.4	10	
LLC	**3**	0.23	84			0	3	
	1			0.12	202	0.23	35	
Blackwater	**2**			0.1	29	0.25	12	
NWR	**3**			0.33	6	0	0	
	1					0.5	107	
Eastern Neck	**2**					0.45	29	
NWR	**3**					0.75	4	
Mean			838		237		253	1328

Table 10 Average percent use of all resources found in winter diet groups (N) of sika deer and white-tailed deer found on the Delmarva Peninsula. Diet samples are averaged across the same study area (alone or with the other deer species), and across years of collection. Dietary resources observed in winter diets of sika deer and white-tailed deer on the Delmarva Peninsula. Resource qualities were indexed based on previous works summarized by the US forest service and the US department of Agriculture. Qualities are based a reference closely related species when the actual resource quality was not available. When multiple qualities are provided for the same resource, we selected the more one that represented the closest geographical area, or quality based on the winter season. Quality for sika deer was based on nutritional values for elk (C. elephus) when provided.

	Mean resource use				**Nutrition value**		
	WTD alone (4)	**WTD W/sika (7)**	**Sika alone (4)**	**Sika W/WTD (3)**	**WTD value**	**sika (elk) value**	**Reference species**
Agrostis gigantea	0.30	1.53	5.47	4.25	1.5	2.5	-
Bromus spp.	4.08	3.10	0.97	3.43	3	3	Bromus tectorum, B. rubens, B. madritensis
Festuca pratensis	3.15	6.49	19.67	10.80	3	3	Festuca rubra
Holcus lanatus	1.93	0.00	0.00	0.65	2	4	-
Hordeum pusillum	2.35	1.61	2.87	7.23	1	2	Hordeum jubatum, H. brachyantherum
Panicum virgatum	0.38	5.03	3.50	1.78	1	1	-
Phragmites australis	0.00	3.04	5.83	6.83	1	1	-

Setaria spp.	0.00	0.00	5.17	4.90	1	1	Setaria parviflora
Spartina patens	0.00	0.63	1.70	0.30	2	2	-
Unknown Grass	1.93	1.71	2.07	1.75	1.7	2.2	-
Carex spp.	0.00	0.77	2.83	4.30	3	3	carex aquaticus
Cyperus spp.	0.00	1.36	5.07	4.85	1.5	1.5	Cyperus esculentus
Juncus spp.	0.00	0.69	2.80	6.75	1.5	1.5	Juncus balticus, J. roemerianus
Scirpus sp.	0.00	0.11	1.87	0.00	1	1	scirpus acutus
Bidens sp.	0.00	0.16	0.00	0.00	2	2	*Bidens aristosa*
Chamaecrista fasciculata	0.00	0.39	0.00	0.00	1	1	-
Legume	0.00	0.21	0.00	0.00	3	3	some great some toxic
Lepidium virginicum	0.00	0.24	0.00	0.00	1	1	Lepidium latifolium, Lepidium draba
Lespedeza virginica	0.00	1.24	0.10	0.30	4	4	-
Melilotus spp.	0.00	0.47	0.00	0.15	5	5	Melilotus alba, M. officinalis
Plantago aristata	2.60	1.49	0.00	0.18	1	1	Plantago rhodosperma
Polygonum punctatum	0.15	1.66	0.57	1.48	4	4	Polygonum biflorum, P. cuspidatum
Unknown Forb	0.30	1.26	0.20	0.60	2.6	2.6	-
Acer rubra	0.68	2.46	0.30	0.00	4	4	-
Amelanchier canadensis	0.30	0.00	0.00	0.00	3	4	Amelanchier alnifolia
Betula nigra	0.68	0.41	0.00	0.00	2	2	-
Carya spp. Leaf	0.58	0.19	0.00	0.00	4	4	Carya laciniosa, illinoinensis
Clethra alnifolia	0.15	10.13	10.70	3.70	1	1	-
Elaeagnus spp. Stem	0.00	0.72	0.00	0.80	2	4	Elaeagnus umbellata, E. commutata
Gaylussacia spp.	4.05	0.40	1.47	0.00	2	2	Gaylussacia baccata

Lonicera spp.	4.73	0.80	0.00	0.00	5	5	Lonicera japonica
Lyonia ligustrina	4.15	3.91	0.10	0.00	1	1	-
Myrica spp.leaf	2.43	8.48	2.00	3.03	1	1	Myrica cerifera
Quercus spp.	9.66	10.83	16.87	15.78	5	5	Quercus alba, Q. rubra, Q. phellos
Rhododendron viscosum	2.63	8.24	0.97	4.53	2	2	Rhododendrom maximum
Rhus copallina	2.28	0.87	0.00	0.00	2.5	1	Rhus glabra,R. copallinum
Rubus spp.	8.35	2.54	0.00	0.00	3	3	Rubus idaeus
Vaccinium corymbosum	9.08	3.47	0.30	2.58	2.5	2.5	Vaccinium arboreum
Unknown Shrub leaf	2.10	2.43	1.27	0.78	2.5	2.6	-
Unknown Shrub stem	0.78	1.89	0.00	1.58	1.4	1.6	-
Unknown Conifer	0.15	0.76	0.00	0.00	1	1	Juniperus virginiana
Pinus spp.	29.75	7.16	4.50	6.35	3	3	Pinus taeda, P. strobus
Unknown Fern	0.00	0.70	0.33	0.40	3	3	Athyrium filix-femina
Unknown Lichen	0.00	0.30	0.00	0.00	1	1	Peltigera aphthosa
Lycopodium obscurum (moss)	0.00	0.00	0.53	0.00	1	1	Polytrichum juniperinum
Unknown Nut	0.00	0.13	0.00	0.00	4	4	-
Unknown Thorn	0.38	0.00	0.00	0.00	1	1	-

Table 11 Means and statistical differences in dietary category use between white-tailed deer (WTD) alone, and when they are sympatric with sika deer, sika deer alone and when they are sympatric with WTD, and sika deer and WTD when they are sympatric with each other.

	WTD Alone - WTD Sympatric				
	Mean Alone	**Mean Sympatric**	**DF**	**T-stat**	**P-value**
Grass	14.1	23.1	9	1.169	0.272
Sedge/Rush	0.0	2.9	6	3.401	0.014
Forb	3.1	7.1	9	2.121	0.063
Woody browse	52.6	57.8	9	0.491	0.635
Conifer	29.9	7.9	9	2.629	0.027

	Sika Alone - Sika Sympatric				
	Mean Alone	**Mean Sympatric**	**DF**	**T-stat**	**P-value**
Grass	47.2	41.9	5	1.020	0.355
Sedge/Rush	12.6	15.9	5	0.949	0.386
Forb	0.9	2.7	5	0.980	0.372
Woody browse	34.0	32.8	5	0.316	0.765
Conifer	4.5	6.4	5	0.121	0.909

	WTD Sympatric - Sika Sympatric				
	Mean WTD	**Mean Sika**	**DF**	**T-stat**	**P-value**
Grass	23.1	41.9	9	2.398	0.040
Sedge/Rush	2.9	15.9	9	4.396	0.002
Forb	7.1	2.7	9	2.281	0.048
Woody browse	57.8	32.8	7	4.565	0.003
Conifer	7.9	6.4	9	0.139	0.893

Table 12 Niche breadth of dietary resource use of white-tailed deer and sika deer from Blackwater National Wildlife Refuge, Tudor Farm and Eastern Neck National Wildlife Refuge as measured via the Shannon-Weiner Information Measure, Levins' Measure of Niche Breadth, and simple count of resources used. Samples from Blackwater are sika deer as the only species. Samples from Eastern Neck are white-tailed deer (WTD) as the only species. Samples from Tudor farm are where both species are in equal relative abundances. All diet groups are 15 individual deer combined. Species listed are single or average of (N) diet groups.

	WTD alone	**WTD with sika**	**Sika alone**	**Sika with WTD**
Resources used (≥5.0%)	18.4 (6.5)	27.7 (6.3)	23.3 (6.5)	25.5 (8.7)
Levins' Breadth (Standardized)	6.066 (0.145)	12.670 (0.271)	9.278 (0.345)	13.200 (0.394)
Shannon-weiner Breadth (Standardized)	2.324 (0.648)	2.875 (0.760)	2.562 (0.796)	2.834 (0.818)

Table 13 Conservative (lowest) indices of Overlap between sika deer and white-tailed deer from separate study areas based on relative abundance of both species (N samples). Groups with high (>.60) degree of overlap from different years were pooled. Each estimate is subscripted with the letter representing that index (P: Pianka's, H: Horn's, S: Schoener's, M:Morisita's).

		WTD Alone (4)	**WTD with sika (7)**	**Sika Alone (3)**	**Sika with WTD (4)**
WTD	**Alone**				
WTD	**with sika**	0.448_{S}			
Sika	**Alone**	0.342_{M}	0.555_{S}		
Sika	**with WTD**	0.370_{M}	0.555_{S}	0.628_{H}	

Table 14 Liberal (highest) indices of Overlap between sika deer and white-tailed deer from separate study areas based on relative abundance of both species (N samples). Groups with high (>.60) degree of overlap from different years were pooled. Each estimate is subscripted with the letter representing that index (P: Pianka's, H: Horn's, S: Schoener's, M:Morisita's).

		WTD alone	**WTD with sika**	**Sika Alone**	**Sika with WTD**
WTD	**Alone**				
WTD	**with sika**	0.764_{H}			
Sika	**Alone**	0.502_{S}	0.752_{P}		
Sika	**with WTD**	0.480_{S}	0.842_{H}	0.888_{P}	

Table 15 Means and statistical differences in diet quality indices created using percent consumption and resource quality. Diet indices are shown between white-tailed deer when they are alone when white-tailed deer are sympatric with sika deer, sika deer when they are sympatric with white-tailed deer, and when sika deer are alone. Significant values at P=0.01 (Bonferroni adjusted for familywise error) are marked with an *.

	Mean 1	Mean 2	df	t-stat	P-value
WTD alone - WTD with sika	280	231	7	4.128	0.004*
WTD alone - sika alone	280	181	5	8.838	<0.001*
WTD alone - sika with WTD	280	183	6	13.651	<0.001*
WTD with sika - sika alone	231	181	8	2.651	0.029
WTD with sika - sika with WTD	231	183	9	3.075	0.013
sika alone - sika with WTD	181	183	5	0.186	0.859

Figure 9 Map of the Delmarva Peninsula highlighting the three study areas used for fecal collections. Areas were selected based on relative abundances of sika and white-tailed deer, habitat similarities and reduced hunting pressure.

Figure 10 Tudor Farms study area with the percentage of fecal pellet groups assigned to white-tailed deer – percent sika deer – and percent that were not assigned and not used in any analysis. Percentages represent multiple collection attempts within the winter along the same transect lines. Pellet groups not assigned, sika deer, and white-tailed deer were observed across the entire study area of Tudor farm, and found within all major habitat types.

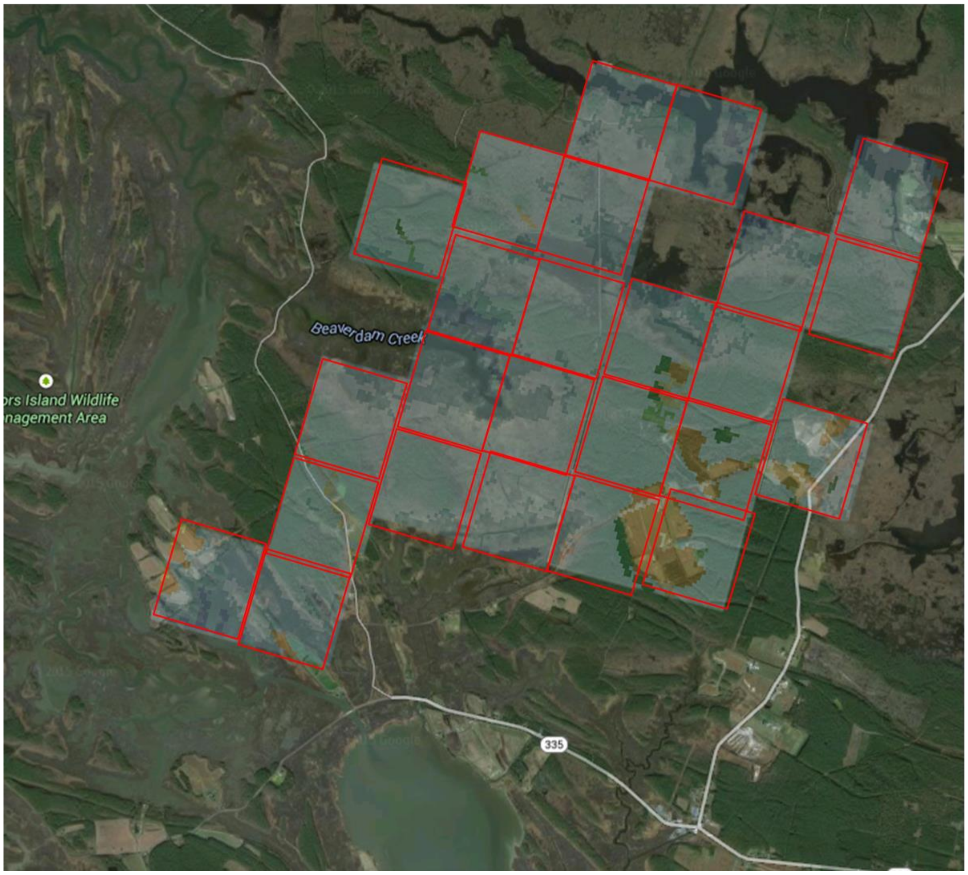

Figure 11 Blackwater National Wildlife Refuge study area and grid of cells. Grid of cells in land use land cover data are overlayed on the arial image of the study area. Cells were layed out on the landscape to avoid open water and maintain a continuous grid on property where we had access.

Figure 12 Eastern Neck National Wildlife Refuge study area and grid of cells. Grid of cells in land use land cover data are overlayed on the arial image of the study area. Cells were layed out on the landscape to avoid open water and maintain a continuous grid on property where we had access.

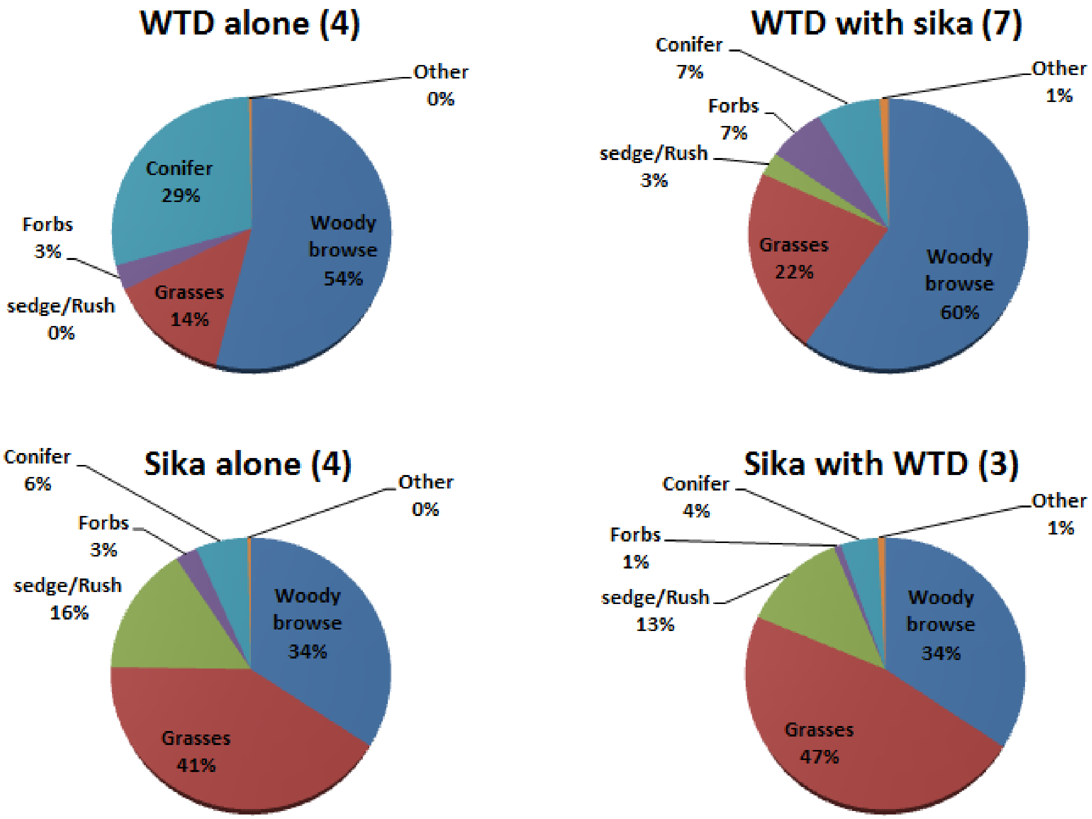

Figure 13 Winter resources used by sika deer (sika) and white-tailed deer (WTD) on the Delmarva Peninsula when alone and sympatric with the other species, from (N) composite dietary groups. All resources for each deer group are listed as dietary categories.

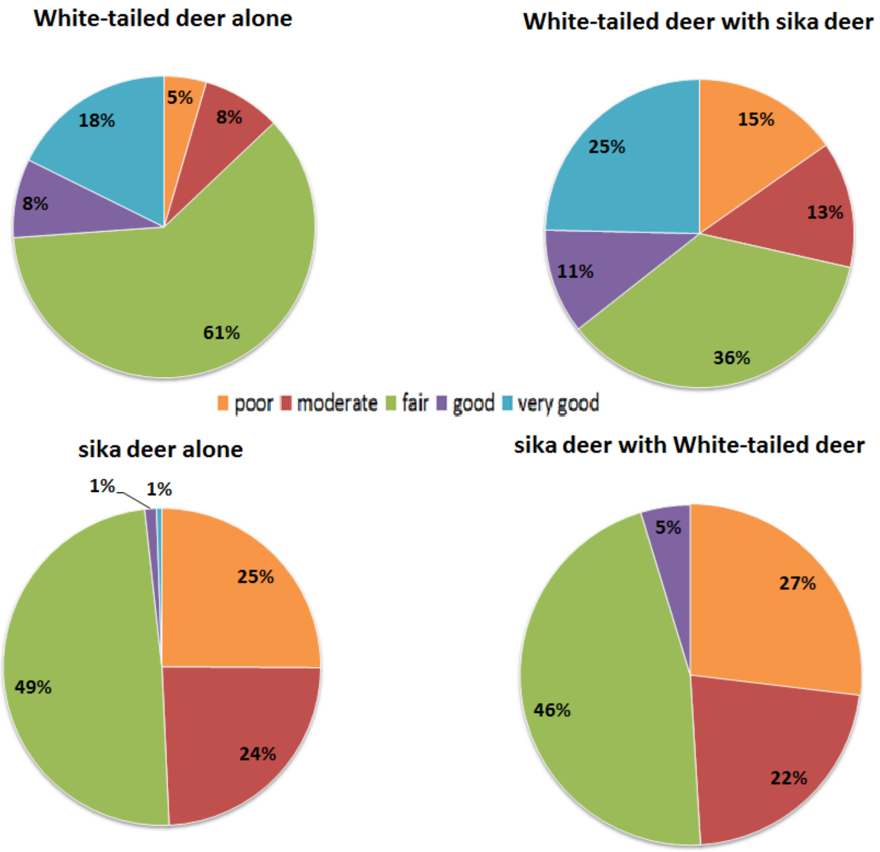

Figure 14 Quality of winter resources used by white-tailed deer, sika deer, and both species while they are sympatric. Quality of forage was based on previous literature from USDA for white-tailed deer and elk (the closest native ungulate). Each resource consumed was provided a quality. Each quality grade is averaged by proportion of use within each deer group.

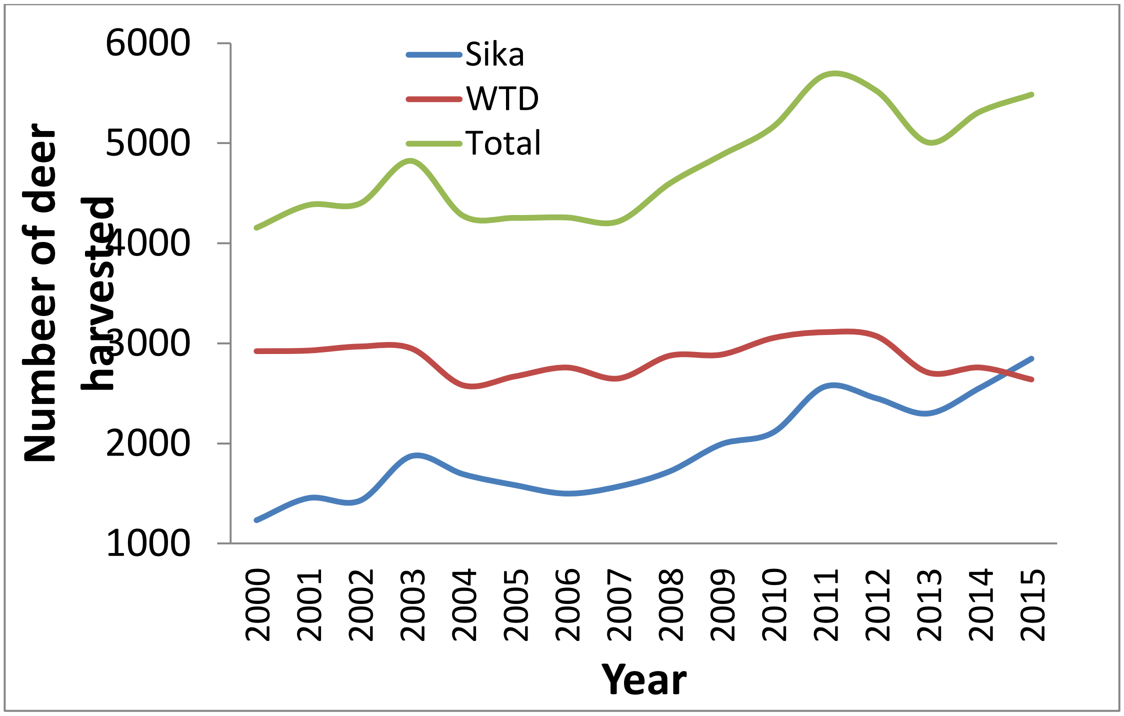

Figure 15 Harvest of deer in Dorchester county Maryland. Percent of sika deer surpassed that of white-tailed deer in 2015.

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Appendix A

FECAL SIZE DIFFERENTIATION

In order to determine if fecal pellets differ in size between sika deer and white-tailed deer, we measured the length, width and height of a single random pellet from each pellet group collected. We calculated a volume per pellet using the 3 measurements. We measured pellets in the field with a microcaliper to the nearest 1/100 mm, and discarded the measured pellet (not used for DNA collection) to prevent contamination from the caliper or from handling. Once we identified the species of the sample through genetic analyses, we grouped the pellets according to species and compared the two groups using an ANOVA in R software base package (R Core Team 2014). We did not use fecal pellet measurements if the species was not confirmed with genetic analysis regardless of where it was collected.

We calculated the volume of 112 white-tailed deer pellets and 67 sika deer pellets. White-tailed deer pellets were larger, $F_{1,177} = 4.42$ $P = 0.037$ with a mean pellet volume of 1252.7 mm^3; mean sika deer pellet size was 1088.5 mm^3. The 1st and 3rd quantile range overlapped with volumes of 800.4 – 1330.6 mm^3 for sika deer and 890.5 – 1486.5 mm^3 for white-tailed deer. These results did not allow us to speciate fecal samples base on measurements.

We were not able to distinguish species based on fecal pellet size. While we did observe a difference in fecal pellets volumes between white-tailed deer and sika deer, the difference was not biologically significant. The difference of an average of 200 mm^3 is so small that it would be hardly noticeable even with measurement tools. Additionally, the observed sizes of pellets, while different as a group, had a high degree of overlap. The wide range in fecal pellet sizes are most likely a result of various age classes of animals being sampled at the same time, in addition to differences in diet and digestion.

Appendix B

IMPACT OF SIKA DEER ON THE SALT MARSH

Introduced deer species can have far reaching detrimental effects that cascade through all trophic levels, down to plant and forest communities (Dolman and Wäber 2008). In addition to the need to manage sika deer for the negative interactions that they pose to white-tailed deer we also should consider the implications to the habitat they reside in. The salt marshes of the Eastern United States are sensitive to changes in herbivory (Silliman and Bertness 2002, Southwick Associates 2004, Hogue and Mouton 2012, Bertness et al. 2014). The addition of sika deer to this sensitive community can further threaten species of concern (National Parks Conservation Association 2007). Sika deer are highly adaptable in their diet and can be aggressively detrimental to a wide variety of vegetation types which can prevent regeneration and alter plant communities (Hannaford et al. 2006, Takatsuki 2009). On Assateague Island sika deer herbivory increased the occurrence *P. australis* (in spite of it being a food resource) which competitively excludes the growth of native vegetation. The impact that herbivores have on salt marshes is dependent on the intensity and the evolutionary history that the grazers have in the habitat (Milchunas and Lauenroth 1993). We observed sika deer diets comprised of up to 80% marsh specific resources, a far greater percentage and more species than native WTD consumed. When addressing changes in saltmarsh communities of Eastern US, we need to critically consider the presence of sika deer, and the potential effects that they can have on the plant life. Climate change and sea level rise are expected to have huge effects on the salt marsh communities (Kern and Shriver 2013, Wiest et al. 2014); coupling these effects with a non-native detrimental herbivore could have compounding effects.

Appendix C

SUPLEMENTAL TABLES

Table 16 Summary of analysis conducted on what groups of samples and the type of genetic information used. A full explanation of each program and the justification for each is found in Methods.

Analysis/Program	purpose	Samples
RFLP with HinF1	speciate fecal samples (WTD or Sika)	All 4 groups, all 109 individuals
GENEPOP	Fisher's exact test for Hardy-Weinberg equilibrium	10 loci and 4 populations (all 109 samples included)
GENEPOP	Dempster's EM method of null alleles and error rates	1409 loci with 283 repeated sample/loci repeats
HP-Rare	rarefication of allelic diversity, private alleles	10 loci and 4 populations (all 109 samples included)

STRUCTURE/HARVESTER	population structure/sample clustering	10 loci and 4 populations (all 109 samples included)
GenALEX	AMOVA – F_{IS},F_{IT}, F_{ST}, PCoA,	10 loci and 4 populations (all 109 samples included)
DIYABC	Estimates bottlenecks, admixture, generational gaps and Ne	10 loci and 3 populations (No Delaware; 97 samples)

Table 17 Sources of Microsatellite information.

Loci	**Reference**
OarFCB193	McDevitt et al. 2009, Senn 2009, Yoshio et al. 2009, Biedrzycka et al. 2012
OarFCB304	Biedrzycka et al. 2012
RT27	McDevitt et al. 2009
OBCAM	Moore et al. 1994
BM4006	Slate et al. 1998, Senn 2009
BM6438	Senn 2009
BM1225	Slate et al. 1998, Kelly et al. 2011
BM4107	Slate et al. 1998, Yoshio et al. 2009
BM203	Slate et al. 1998, Goodman et al. 2001, McDevitt et al. 2009, Yoshio et al. 2009, Biedrzycka et al. 2012
IGF-1	Kelly et al. 2011
RM188	Slate et al. 1998, Senn 2009, Biedrzycka et al. 2012
TGLA126	Slate et al. 1998, Senn 2009
TGLA127	Slate et al. 1998, Senn 2009
TGLA337	Slate et al. 1998, Senn 2009
IDVGA55	Slate et al. 1998, Senn 2009, McDevitt et al. 2009

Table 18 Allele frequencies observed in sika deer in Scotland by Senn 2009 and those observed in this study across the same loci. Alleles TGLA126, TGLA127, and IDVGA55 were tested for this study and did not perform well so were excluded from all analysis; they are included in this table as anecdotal information with sample sizes. Similar allele frequencies for these loci but different allele sizes may be a result of differences in scoring techniques.

Locus	**Allele**	**Senn 2009 Scotland (735)**	**Kalb Maryland (83)**	**Kalb** Yakushima **Japan (14)**	**Kalb Delaware (12)**
BM4006	**83**	NA	0.000	0.036	0.000
	85	0.946	0.000	0.000	0.000
	87	0.000	1.000	0.894	0.625
	89	NA	0.000	0.000	0.375
	93	0.053	0.000	0.000	0.000
	95	0.000	0.000	0.000	0.000
	Null	0.001	0.000	0.070	0.000
BM6438	**251**	0.009	0.000	0.000	0.000
	263	NA	0.000	0.077	0.000
	265	0.241	0.000	0.039	0.000
	267	NA	0.000	0.000	0.408
	275	0.747	0.000	0.000	0.000
	277	NA	0.988	0.846	0.058
	281	NA	0.000	0.039	0.117
	Null	0.002	0.012	0.070	0.583
FCB193	**103**	0.021	0.000	0.000	0.000
	107	0.000	0.000	0.072	0.000
	111	0.000	0.000	0.036	0.000
	121	NA	0.000	0.000	0.042
	123	NA	0.000	0.072	0.125
	125	NA	0.988	0.715	0.208
	129	NA	0.000	0.000	0.375
	131	NA	0.000	0.000	0.083
	132	0.977	0.000	0.000	0.000
	141	NA	0.000	0.036	0.000
	Null	0.001	0.012	0.07	0.167
IDVGA55	**195**	0.005			
	199	0.005			

	210	0.923	
	212	0.066	
	215	NA	1.00 N= 28
	Null	0.001	
TGLA126	**100**	0.402	
	101	0.576	
	105	0.019	0.25 N= 25
	Null	0.003	0.75
TGLA127	**161**	0.877	
	174	0.095	1.00 N= 23
	178	0.025	
	Null	0.001	