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NON-INVASIVE GENETIC SAMPLING IN THE ANALYSIS OF WHITE-TAILED DEER POPULATION CHARACTERISTICS

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By

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SIGNATURE APPROVAL FORM

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ABSTRACT

NON-INVASIVE GENETIC SAMPLING IN THE ANLAYSIS OF WHITE-TAILED DEER POPULATION CHARACTERISTICS

By

Grant Montgomery Slusher

I evaluated the use of non-invasively collected hair samples from white-tailed deer (*Odocoileus virginianus*) in three contexts. First I assessed the effects of sampling interval and barb location on the probability of sample cross-contamination of hair snares. Six hair snares were installed on Presque Isle Park, Marquette Michigan from 11 May 2008 to 3 July 2008 and hair was collected from each daily. Probability cross-contamination increased from 12% to 28% during sampling intervals of two and seven days, respectively, but was unaffected by barb location. Second I assessed the benefits and costs of using non-invasive techniques for population estimation. I estimated abundance of the Presque Isle deer herd from genotype data derived from barbed-wire snared hair, and compared these estimates to drive counts performed during this study. Genotype-based estimates were greater than the drive counts, probably due to the genetic "capture" of animals that frequently move on and off the peninsula. This represents the first successful use of non-invasive genetic sampling for population estimation of ungulates. Third, I compared the amount of genetic differentiation and migration between two island-mainland systems in Lake Superior. Results differed from a standard biogeographical prediction that populations on larger islands closer to their mainland source population should have higher indices of genetic connectivity. The results of this research should be used to inform future studies that use non-invasive genetic sampling for ungulate population research.

This thesis is dedicated to my family.
Their support is the only way I could have survived academia this long.

ACKNOWLEDGEMENTS

First, without funding this research would not have been feasible. The Christine Stevens Wildlife Fund from the Animal Welfare Institute provided a majority of the funding for this project. Also, the Charles C. Spooner and Excellence in Education grants were essential in stocking the Lindsay Lab shelves, and purchasing reagents for Athena.

Snare installation would have been impossible without a second pair of hands in the field. I would like to thank Eric Palm, Kim Longhini, and Danielle Hernandez for their volunteer efforts. While the elements and terrain were difficult on some days (to say the least) they showed character in lending me some of their spare time. Besides my praise I hope they at least gained experience to fill out a CV.

It is always nice to be surrounded by a group of misfits experiencing the same trials and tribulations as yourself. With that said the graduate student body of the biology department provided excellent sounding boards, office mates, field assistants, lab assistants, drinking partners, and hiking pals. I congratulate all those before me and wish those to come the best of luck. Science requires patience and persistence and your peers have the outside perspective needed to remind you of the light at the end of the tunnel.

Dr. Alan Rebertus was my link to the world of statistics. Chapter 1 was a result of his patience in watching me fumble through PASW for a simple logistic regression. Dr. Jerry Belant provided the infield snare installation training and expertise in the field of non-invasive genetic sampling needed for completion. Referring back to my fumbling with statistical analyses, Dr. Bruggink's aid in building MARK models was critical for deriving any type of population

estimate for Presque Isle deer. It takes more than savvy with .txt files to interpret an entire capture-mark-recapture data set. The quirks of such software would have taken me a lifetime (or at least a PhD) to understand. Dr. Kate Teeter's expertise and experience with microsatellites prevented me from giving up early on during the laboratory portion of this study. Those two qualities are a small fraction of her contribution as her easy going demeanor created the perfect environment for asking questions (as simple as they may have been). Also she has the nicest molecular lab equipment a fledgling geneticist could ask for (sorry Bonnie and Clyde, Theo's so shiny and new). My thesis committee chair and advisor, Dr. Alec Lindsay gave me a chance to practice science. The countless canned laboratories and informal lab reports of an undergraduate only provide a set of motions to go through. Applying a model, establishing a method, and answering a question require ingenuity and persistence. When I was offered this project I will admit I had no idea what I was getting into. Fortunately, my advisor was patient and offered words of encouragement especially when it came to refining my own writing. I appreciate the time each of my committee members has given me.

Finally the Dunbar and Slusher clans have shown the most patience of anyone. When my resolve deteriorated they never questioned whether or not I would complete the work (even when I did). Though they may not know it, the question "So when will you graduate?" is the best motivation to any college student. While I was tired of answering (in all honesty I barely knew when I would graduate) it was a reminder to finish what I had started. I'm lucky to have this ultimate support group.

The Application to use Vertebrate Animals (Application#IACUC 076) was approved by the Animal Welfare Care and Use Committee on January 11, 2008. The approval period spanned from May 1, 2007 to May 1, 2009. This thesis is formatted for submission to Molecular Ecology.

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CHAPTER 1: A TEST OF HAIR SNARE SAMPLING PROTOCOLS

Introduction

Conservation studies of wildlife depend on robust population estimates which themselves must accurately sample and identify individuals. Population size estimation often employs a capture-mark-recapture technique that allows statistical extrapolation of total population sizes from a subsample of individuals from that population. Researchers can use passively collected tissues as a source of DNA to minimize interactions between researchers and animals. (Phillips *et al.* 1993; Kohn *et al.* 1999; Mills *et al.* 2000). Passive genetic sampling techniques rely on the collection of hair, feathers, or scat as a source of DNA (Woods *et al.* 1999; Downey *et al.* 2007; Ruell & Crooks 2007). Three major benefits of noninvasive genetic sampling techniques are: 1) they reduce stress to animals resulting from contact with researchers 2) the genetic profiles created as "tags" cannot be lost by the animal, and 3) the genetic profiles of individuals in a population provide information regarding the life history of animals in a population (Conner *et al.* 1987; DeNicola & Swihart 1997). Therefore noninvasive genetic sampling and tagging offers an alternative means of population estimation and allows researchers to further investigate meaningful population genetic parameters like inbreeding coefficients and population structure (Morin *et al.* 1993)

While scat and hair have been used as non-invasively collected sources of mammalian tissue, fecal DNA has proven problematic in analysis due to PCR inhibitors and a higher probability of degradation (Fernando *et al.* 2003; Nsubuga *et al.* 2004;). Hair snares are another way to collect genetic tissue samples (McDaniel *et al.* 2000; Sloane *et al.* 2000; Beier *et al.* 2005). Baited hair snares can reduce sampling effort by attracting animals to sampling sites where

hair is passively collected (McKelvey & Schwartz 2004). Hair snares have been successfully used to sample a variety of mammalian species, including lynx (*Lynx canadensis*), hairy-nosed wombat (*Lasiorhinus krefftii*), and brown bear (*Ursus arctos*) (Beier *et al.* 2005; McDaniel *et al.* 2000; Sloane *et al.* 2000, respectively). The basic technique of using hair snares with large mammals involves luring animals to a barbed wire enclosure with an attractant (Mowat & Strobeck 2000; Belant *et al.* 2005). Animals approach the attractant and contact the barbed wire, leaving clumps of hair behind on the wire barbs. Field technicians can collect hair clumps from the wire barbs and use those hair clumps for later genetic analyses. Genotypes established from those hair samples can be used to estimate population sizes (Boulanger *et al.* 2004; Beir *et al.* 2005; Belant *et al.* 2005; Bellemain *et al.* 2005) through standard capture-mark-recapture methods (see Chapter 2).

Although non-invasive genetic sampling provides some benefits to wildlife studies, there are potential drawbacks that differ from drawbacks normally associated with traditional capture-mark-recapture protocols. In particular, if genetic identification procedures are flawed, resulting population estimates will be incorrect. There are two potential causes for genetic misidentification: genotyping errors and hair sample cross-contamination. Genotyping error rates can be accounted for (see Chapter 2 for example), but cross-contamination of hair samples can create problems before genotyping takes place (Waits & Paetkau 2005). For instance, hair clumps containing tissue from more than one individual can return genotypes with three or more alleles per locus, or more problematically, they could make a homozygote look heterozygous. One way to avoid multiple-individual hair clumps is to use only single hairs for genotyping. However, past studies demonstrated that single hairs generally do not provide adequate templates for genotyping (Goossens *et al.* 1998; Sloane *et al.* 2000). The most reliable way to prevent cross-contamination

during DNA amplification from snared hair samples is to collect every hair sample immediately after it is snared. The trade-off for increased assurance of non-contamination is a concomitant increase in cost for field collection, diminishing the benefit of using passively collected DNA samples.

My objective was to evaluate three questions regarding the collection of hair samples for studies of white-tailed deer (*Odocoileus virginianus*) that use non-invasive genetic sampling techniques: 1) what visitation frequency minimizes or eliminates cross-contamination of hair-snare tissue samples, 2) do positional characteristics of barbs affect their probability of becoming cross-contaminated over time, and 3) what are the time and funding costs/benefits associated with increased sampling efforts at hair snares? Regarding researcher visitation frequency, I predicted that increasing the length of time between the researcher sampling visits would increase the probability of cross-contamination on snares. As to positional characteristics of barbs, since hair snares in most studies are designed with either a triangular or square shape around a lure pile (Belant *et al.* 2005; Belant *et al.* 2007), medial barbs of a snare line will be closer to the lure than barbs at the distal corners of the snare. I thus predicted that the frequency at which barbs are contacted by animals should decrease from medial to distal barbs on a snare line. Finally, I provide a cost-benefit analysis of the trade-offs that are associated with snare visitation schedules of varied levels of intensity.

Materials and Methods

Presque Isle Park of Marquette Michigan, USA is a 1.31-km^2 (46º35’09.71”N, 87º22’55.75”W) peninsula located at the northern edge of the city of Marquette. Presque Isle Park includes hardwood forest types composed of maple (*Acer* spp.) white-birch (*Betula papyrifera*), and ironwood (*Ostrya virginiana*) with intermittent stands of white-pines (*Pinus strobus*) and eastern hemlock (*Tsuga canadensis*). Since April 1999 a population of white-tailed deer (*Ococoileus virginianus*) has ranged from 8 to 100 individuals on the peninsula (J. Bruggink, *unpublished data*).

Fig. 1. Map of Presque Isle Park of Marquette, MI with snare locations. © Google 2010

I installed six snares on Presque Isle Park on 11 May 2008. To evenly space snares across the study area I superimposed a rectangular grid composed of four 0.35 km by 0.20 km cells over a GoogleEarth© (Google Inc.) image of Presque Isle Park (Fig. 1). The grid was centered inside of the paved road that circumnavigates the park perimeter. I identified UTM coordinates of cell corners and then selected specific snare locations near these corners with evidence of deer and adequate groupings of trees (Fig. 1).

Using fencing staples, 15.5 gauge four-pronged barbed wire was attached to 3-4 trees about 70 cm above ground creating a polygon (Belant *et al.* 2007). In areas of uneven ground, soil was added or removed to maintain 70 cm height of the barbed wire. One liter of Buckjam© (Evolved Habitats) was poured over a small pile of sticks in the center of the enclosure and reapplied at two-week intervals. Information signs were stapled to each anchor tree facing out from the center of the snare.

Deer hair was collected daily from barbs, beginning one day after installation (12 May 2008) using a pair of flame-sterilized forceps and hair clumps were deposited into individually labeled 1.5mL micro centrifuge tubes. Date, snare number and barb number were recorded for each sample. A butane-lighter was used to burn away remaining tissue on barbs to prevent cross-contamination of new samples on previously-used barbs. Collection continued until 17 June 2008, after which all snares and signage were removed. All procedures were approved by Northern Michigan University Institutional Animal Care and Use Committee (IACUC #076) and the Marquette Parks and Recreation Committee.

Daily collection of samples from each barb allowed evaluation of the probability of cross-contamination on single barbs over different intervals of time. Twenty five of the 310 barbs snared hair with a high enough frequency that they would have had sample cross-contamination if they had they been left without sample collection for seven days. These 25 barbs were the only ones included in this analysis. To determine the probability of a barb collecting hair from more than one individual within various sampling intervals, I used PASW 17.0 (SPSS Inc.) to perform a binary logistic regression with forward conditional model selection and Hosmer-Lemeshow *post hoc* test for goodness of model fit. Zero represented no cross-contamination event and one represented a cross-contamination event. The dependent variable was the probability of cross-

contamination and the independent variables were sampling interval, barb location relative to center (a ranking of one being central and increasing rankings indicating more distal barbs), and snare.

Results

The six snare set-ups across the study area contained a total of 310 barbs. Of these 310 total barbs, 105 collected at least one hair clump and 51 barbs collected at least two hair clumps (Fig. 2). Twenty-five barbs collected at least two hair clumps within seven days, and these were used to assess potential cross-contamination if un-sampled periods had lasted seven days.

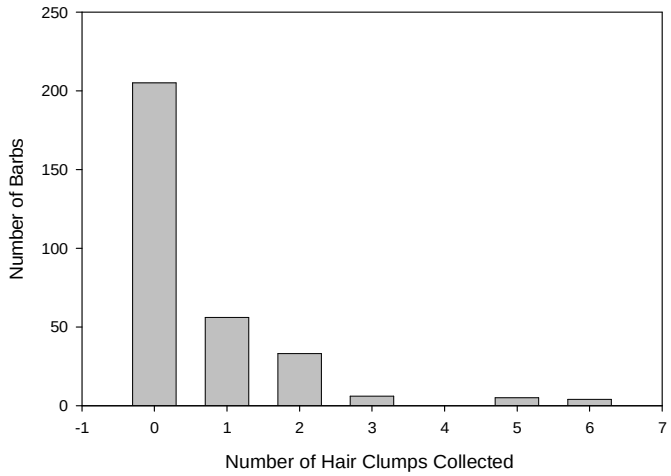

Fig. 2. Frequency histogram showing the number of barbs that collected different numbers of hair clumps. (SigmaPlot 9.0 © Systat Software Inc.)

All hair clumps collected from any barb on a single day were assumed to be from single individuals. The probability of cross-contamination increased with sampling interval ([1/1+$e^{-(-}$

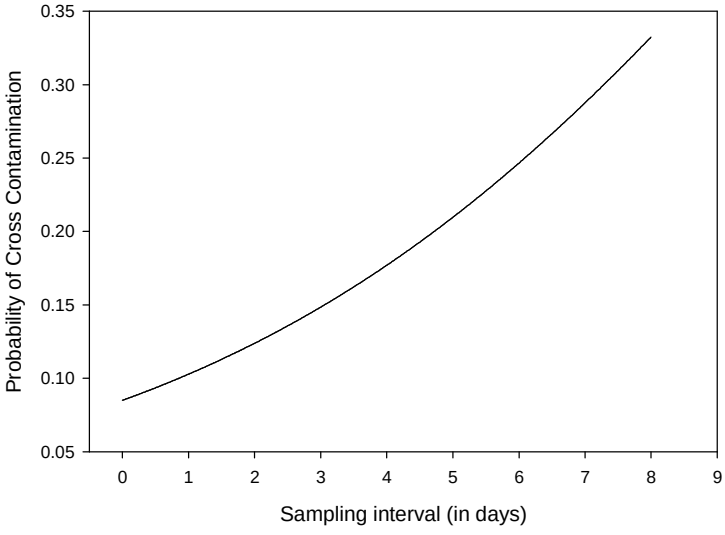

Fig 3. Probability of cross-contamination shown as a function of sampling collection interval. (SigmaPlot 9.0 © Systat Software Inc.)

$^{2.337+0.210*\text{sampling interval})}$], $P < 0.001$) while barb location ($P = 0.874$) and snare ($P = 0.290$) did not improve model fit. Data did not significantly deviate from the best-fitting model ($X_4^2 = 2.002$, $P = 0.735$). The relationship between the probability of cross-contamination and the sample collection interval was found to be significant ($X_1^4 = 14.836$ $P < 0.001$). Probability of cross-contamination increased from 12.4% on day 2 to 28.8% on day 7 (Fig. 3).

Discussion

Choosing snare locations and timing intervals for sample collection depends on study goals. In this study, snares had varying degrees of tissue collection success, but snare location did not influence probability of cross-contamination. Location of certain snares relative to preferential habitat caused them to collect more tissue than others. Variation between individual snare tissue-collection went undetected by the logistic regression analysis. Barb location relative to the bait pile also had no significant effect on the probability of cross-contamination, showing that distal barbs were just as likely to experience cross-contamination as medial barbs.

Minimizing genotyping errors in hair snare studies of wildlife species is important (Taberlet & Luikart 1999; McKelvey & Schwartz 2004). Genotyping errors increase with decreasing numbers of hairs. For example, Goossens *et al.* (1998) found genotyping errors increased from 0.3% to 4.9% to 14% when DNA was extracted from ten, three and one hairs of alpine marmots (*Marmota marmota*), respectively. Genotyping errors also arise from the use of hair clumps with tissue from more than one individual (Sloane *et al.* 2000; Waits & Paetkau 2004). Balancing these two error rates means balancing effort and resources invested into single hair extractions against increasing snare collection visits (Taberlet *et al.* 1996).

In this study, time and resource investments for genotyping analyses were greater than the investments required by field collection activities. A single sample collection visit to all snares on

Presque Isle took 1.5-2.0 hours every day during the collection period. In contrast, extraction of DNA and genotyping took seven hours per sample. When snares were visited every day, a maximum of 14 hours were dedicated to field collection per week and 48 hours were dedicated to extraction and genotyping of samples. By my collection model, daily visits would still result in 12% cross-contamination of samples, but these would hopefully be identified in the genetic analysis through the appearance of spurious three-allele genotypes at some loci (an impossibility in a diploid organism, and therefore indicative of a contaminated sample).

If snares were sampled only once a week, the investment in field collection would be reduced from 14 to two hours per week. To ensure non-contamination of collected hair samples, genotypes could be based on single-hairs DNA extractions as suggested by Goossens *et al.* (1998). This would require an initial 48 hours of laboratory genotyping analysis, but single-hair genotyping has a 14% error rate. To provide confident genotype data from single-hair extractions, re-amplification is required (Goossens *et al.*1998), which would conservatively require an extra three hours of amplification and 12 of visualization. Ultimately the 12 hours saved in the field is nullified by the extra 15 hours spent in the lab. Using multi-hair samples will reduce the need for extensive re-amplification of samples. Researchers can more confidently use multi-hair samples if the time between collections from a hair snare is shortened significantly enough to prevent cross-contamination. This study showed no incidents of potential cross-contamination on successive days (no barbs had hair clumps on successive days) so at a minimum, an every-other-day collection interval should create a low probability of cross-contamination. Furthermore, many commercial labs that perform genotyping analyses require five or more hairs to reduce the effort required to genotype each sample (Wills 2008). Agencies that intend to use those labs for genetic analysis should obviously consider adopting field protocols that will reduce cross-contamination.

The relatively high density of white-tailed deer on Presque Isle (averaging 22 deer/km^2 yearly since 1999) means that these estimates of cross-contamination are likely significantly higher than what would occur in more normal populations where the average deer density is 12 deer/km^2 (Smith 1991). Since the density of deer on Presque Isle nearly doubles that of other populations in similar habitat types, researchers wishing to minimize cross-contamination of hair snares should consider that white-tailed deer activity in lower-density populations would likely be lower than the rates described here. Populations of a higher density will require more snares to detect all individuals (Boulanger *et al.* 2004). Studies of wild black bear (*Ursus americanus*) populations that use hair snares for non-invasive genetic sampling have successfully avoided cross-contamination using 10-day sampling intervals when bear densities are below one bear/km^2 (Bellemain *et al.* 2005). Understanding that most populations of deer and other ungulates are less dense than the population used in this study means that applying sampling intervals of two days or greater could still effectively prevent significant sample cross-contamination.

Conclusion

The use of non-invasive genetic sampling is increasing in the fields of population ecology, conservation genetics and wildlife management. Hair snares are commonly used to passively collect genetic samples from mammals, but the costs and benefits of varying snare visitation by researchers have remained unknown, especially for ungulates. For sampling the relatively dense population of deer on Presque Isle the snare location relative to preferential habitat had no significant effect over the probability of cross-contamination. Barb location relative to the lure can also be ignored as a contributor to increasing cross-contamination probabilities. To diminish the probability of cross-contamination of hair samples, field collection protocols should decrease the time between snare collection visits. With this study, 12% of multi-hair samples could have

experienced cross-contamination if collected from snares every two days. This study suggests that increasing field effort can greatly diminish the costs that would otherwise be required if genotyping is being performed based on the more error-prone single hare analysis (Goossens *et al*. 1998). In considering these cross-contamination rates to the design of studies on other populations, protocols should obviously consider other important characteristics like the preferential habitat use of the study species and the population density of the specific area under investigation. The density of the population examined in this research is unusually high (22 deer/km^2) compared to more normal white-tailed deer population densities (Smith 1991). For a sparser population of white-tailed deer two- or three-day sampling intervals could effectively reduce cross-contamination at snares. This is further supported by hair snare studies of bears where cross-contamination was absent when sampling every ten days in population densities of less than one bear/km^2.

CHAPTER 2: COMPARING NON-INVASIVE GENETIC SAMPLING POPULATION ESTIMATES TO A DIRECT DRIVE COUNT OF WHITE-TAILED DEER

Introduction

Non-invasive genetic sampling (NGS) paired with capture-mark-recapture (CMR) modeling can provide benefits not realized with traditional capture-recapture techniques (Mowat & Paetkau 2002; Belant *et al.* 2005; Soldberg *et al.* 2006). Traditional CMR methods require considerable investments of labor and time to tag and track individual animals. These investments can be reduced in NGS studies that use passive collection of tissue samples for DNA fingerprinting (Kohn *et al.* 1999; Boulanger *et al.* 2004). Non-invasive genetic sampling techniques may be less costly for researchers, and may be less prone to other technical pitfalls of traditional CMR studies (e.g., researcher-animal interaction, loss of tag or radio collar; Woods *et al.* 1999). The reduction in field time and effort and assignment of permanent individual identifiers (i.e, genotypes) are advantages of noninvasive genetic techniques in studies of wildlife populations.

However, NGS studies of wildlife populations are also prone to pitfalls not associated with traditional CMR studies. Although behavioral variability of animals can positively or negatively bias population estimates (Otis *et al.* 1978), genotyping errors are perhaps the most odious of possible problems for CMR studies that depend on genetic data. An assumption in CMR modeling is that animals are marked and recorded accurately during each sampling session (White 1982). Analogous to the accidental failure to mark a caught individual, if a genetic sample

fails to be genotyped accurately for some reason, that animal will go unmarked. The "marks" used in a genetic "capture" methodology are the microsatellite genotypes of individual animals, and this allows for the possibility of genotyping errors to lead to different individuals being identified (and thus "marked") with the same genotype across a given set of loci. Indeed each individual (other than identical twins) should have its own unique genotype, but it is possible that the loci sampled from two different individuals would have the same alleles at the limited number of loci sampled in the study. These duplicate genotypes (called "shadow" genotypes) can arise during a microsatellite study because of low variability of markers or from a panel composed of too few markers (Paetkau 2003; McKelvey & Schwartz 2004).

The probability of identity ($P_{(ID)}$) gauges the diagnostic and analytical power of the marker set used for a population by describing the chance that two individuals in a population will share identical genotypes (Waits *et al.* 2001; Valière 2002). The $P_{(ID)}$ value for a set of microsatellite markers indicates whether or not the analysis will resolve individuals of a population or if more variable markers are needed. After determining that a marker set has acceptable $P_{(ID)}$ values ($\leq$0.0001) researchers can then proceed with CMR modeling with confidence that multiple individuals will not share a genotype (Valière 2002).

Allelic dropout is a different type of analytical problem that arises when one allele at a locus is sporadically unamplified in polymerase chain reaction (PCR)-based analyses. For example, if the same individual is sampled twice, but PCR analysis of one sample of the individual happens to suffer from an allelic dropout event, then the two samples will appear to be from two individuals that differ by one allele. This is a clear violation of the assumption made during CMR modeling that no misidentifications are made during capture and recapture. Most commonly, when allelic dropout occurs and is detectable, a heterozygous genotype is

misidentified as a homozygote (due to the "dropout" of one allele). The appearance of this "new" homozygote in the data set will inflate the number of first time captures, while simultaneously reducing the number of recaptures. A high number of new "marks" will inflate the population estimate and broaden confidence intervals as the recapture of marked individuals decreases (Paetkau 2003; McKelvey & Schwartz 2004; Lukacs & Burnham 2005).

Non-invasive genetic sampling methods have been applied to coyote (*Canis latrans*), black bears (*Ursus americanus*), and brown bears (*Ursus arctos*) (Goossens *et al.* 1998; Belant *et al.* 2005; Bellemain 2005). With each genetic capture-mark-recapture study researchers passively collected either feces or hair as a source tissue. Each sample was then assigned a date of collection and a location of origin. Ultimately each study concluded with an estimate of population density for the specified area. For example Kohn *et al.* (1999) estimated 38 coyotes (CI = 36 – 40) for a region of the Santa Monica Mountains, CA. Using the date of collection and global positioning system coordinates, Kohn *et al.* (199) used genotyped scat to perform CMR analysis for this estimate. Hair snares provided a means of passively identifying individual black and brown bears with genotypes, which were subsequently used to estimate population sizes and densities in different geographic regions (Belant *et al.* 2005; Bellemain *et al.* 2005).

Although NGS has been used to estimate carnivore population sizes, only one study has successfully evaluated this method as a tool to estimate ungulate populations (Ebert *et al.* 2010) . My objective was to use noninvasive genetic sampling to estimate the size of a white-tailed deer (*Odocoileus virginianus*) population that is regularly monitored through drive counts. My design used noninvasive hair snares to collect tissue from individual deer and then assemble capture histories for incorporation in a capture-mark-recapture model. I then compared my population estimate to drive counts of the population. The counts provided a means of validating my

estimates and determining whether or not noninvasive genetic sampling can be effective in estimating population sizes.

Materials and Methods

Presque Isle Deer Population

Presque Isle Park of Marquette Michigan, USA is a 1.31-km^2 (46º35'09.71"N, 87º22'55.75"W) peninsula located at the northern edge of the city . Presque Isle Park includes hardwood forest types composed of maple (*Acer* spp.) white-birch (*Betula papyrifera*), and ironwood (*Ostrya virginiana*) with intermittent stands of white-pines (*Pinus strobus*) and eastern hemlock (*Tsuga canadensis*). Since April of 1999 a population of white-tailed deer (*Ococoileus virginianus*) has ranged from 8 to 100 individuals on the peninsula (J. Bruggink, *unpublished data*).

Fig. 4. Snare locations on Presque Isle Park, Marquette, MI. © Google 2010

I installed six snares in Presque Isle Park before two sampling sessions: 8 February 2008 to 11 April 2008 and 11 May 2008 to 17 June 2008. For the February 2008 session I installed snares in places intended to avoid interference from the public and intended to make them easily accessible by footpath. For the May 2008 session I evenly spaced snares across the study area by superimposing a rectangular grid composed of four 0.35km by 0.20km cells over a GoogleEarth©

(Google Inc.) image of Presque Isle Park (Fig. 1). The grid was centered inside of the paved road that circumnavigates the perimeter of the park. I identified UTM coordinates of each of the lateral corners of the four cells and used them to identify each site in the field. I then selected specific locations based on sign or presence of deer and whether there were adequate groupings of trees (Fig. 4).

I used fencing staples to attach 15.5 gauge four-pronged barbed wire to 3-4 trees about 70 cm above ground to create a polygon around a scented lure (Belant *et al.* 2007). In areas of uneven ground, soil was added or removed to maintain a consistent 70 cm height for the barbed wire. I poured one liter of Buckjam© (Evolved Habitats) was poured over a small pile of sticks in the center of the enclosure as a lure, and refreshed it at regular two week intervals. Bright yellow information signs were stapled to each anchor tree facing out from the center of the snare.

I collected deer hair from barbs every two to three days for the February 2008 session and daily for the May 2008 session beginning one day after installation. Hair clumps were collected from barbs using a pair of flame-sterilized forceps and then deposited into individually labeled 1.5mL micro centrifuge tubes. Date, snare number and barb number were recorded for each sample. A butane-lighter was used to burn away remaining tissue on barbs to prevent cross-contamination of new samples on previously used barbs. Collection continued until 17 June 2008, after which I removed all snares and signage. All procedures were approved by Northern Michigan University Institutional Animal Care and Use Committee (IACUC #076) and the Marquette Parks and Recreation Department.

DNA Extraction and Microsatellite Amplification

Collected samples were stored at 4˚C until DNA extraction was performed using DNEasy Tissue Kits® (Qiagen) according to manufacturer's directions. DNA was suspended in the AE

buffer and stored at -20°C until PCR amplifications. Genotyping of the DNA samples was performed using a suite of loci that consisted of: two loci derived from mule deer (*Odocoileus hemionus*: *OhD, OhN*; Paetkau unpublished), two loci derived from domestic cattle (*Bos taurus*: *BM4107, BM6506*; Bishop *et al.* 1994), and one locus designed for caribou (*Rangifer tarandus: RT24*; Wilson *et al.* 1997). PCR amplifications were performed using Bullseye HS Taq (Midsci) and a three-primer CAG-tailing system (Schuelke 2000). Amplification of each sample was performed in 15µL reaction volumes containing 10-50ng of genomic DNA template, 1X HS Buffer II (with 2.0mM $MgCl_2$), 0.2mM of dNTPs, 0.2mM forward primer with CAG tail, 0.5mM fluorescently labeled CAG primer, 0.7mM reverse primer and 0.5 units *Taq* polymerase (Schuelke 2000). A negative control was included with each set of reactions to identify potential contamination of the mastermix. The three fluorescently labeled primers used in the three-primer CAG-tailing system were 6-FAM, PET, and VIC (Applied Biosystems).

Loci *OhD, OhN*, *BM4107*, and *BM6506* were amplified using the following thermoprofile: 15 minute hot start at 95˚C followed by: five cycles of 95˚C for 15 seconds, 55˚C for 30 seconds, and 72˚C for 1 minute. This was followed by a 6-cycle annealing temperature touchdown sequence, where annealing temperatures were dropped from 54˚C to 48˚C in one-degree decrements. The following 29 cycles consisted of a 95˚C for 15 seconds, 48˚C for 30 seconds and 72˚C for 1 minute. A final elongation at 72˚C for five minutes was performed before storing samples at 4˚C. The fifth locus, RT24, was amplified using the following thermoprofile: a hot start at 95˚C for 15 minutes followed by; ten cycles of 95˚C for 15 seconds, 58˚C for 30 seconds, and 72˚C for 1 minute. This was followed by a 4-cycle annealing temperature touchdown sequence where the annealing temperatures were dropped from 57˚C to 53˚C in one-

degree decrements. The final 34 cycles included denaturation at 95˚C for 15 seconds, annealing at 53˚C for 30 seconds, and elongation at 72˚C for 1 minute. All PCR reactions were run either in a MasterCycler® Gradient thermocycler (Eppendorf) or MyCycler® (Bio-Rad) thermocycler.

Genotype Scoring

Alleles at all loci were sized using polymer-based electrophoresis on an ABI 3100-*Avant* Genetic Analyzer equipped with a 50cm capillary array and POP-6 polymer (Applied Biosystems). Loci were divided into two different panels for allelic sizing by the ABI 3100-*Avant* Genetic analyzer: PanelA consisting of *RT24*/6-FAM, *BM4107*/VIC, *BM6506*/PET and PanelB consisting of *OhD*/VIC, *OhN*/PET. Amplification products were loaded onto the ABI 3100-*Avant* Genetic Analyzer in multiplex cocktails. Cocktail panels were created by mixing 5µL of the amplification products from each individual locus in a new tube. A small amount (1µL) of this multiplex mixture was combined with 11.5µL of Hi-Di Formamide and 0.5µL LIZ-600 internal size standard (Applied Biosystems). Before loading onto the genetic analyzer the mixture was heated to 95˚C for two minutes cooled at 4˚C until subjected to electrophoresis. Allele sizes were scored using GeneMapper 3.5 (Applied Biosystems), which compares migration distances of fluorescently labeled amplicons to migration distances of known LIZ-600 (Applied Biosystems) size standard fragments. Fragment sizes were recorded as basepair lengths. After initial basepair sizes were assigned, electropherograms were visually inspected to reconcile poor size calls, and to mark fragment peaks that were initially ignored during GeneMapper analysis. The sex of each sample was determined using a PCR-based protocol with *CerZFXYf* and *CerZFXYr* primers (Lindsay & Belant, 2008).

Summary Statistics

The frequency of null alleles (non-amplified "dropout" alleles) per locus was estimated using Micro-Checker (Van Oosterhout *et al.* 2004). Loci with high rates of estimated null allele occurrence were used to evaluate the apparent uniqueness of individual genotypes. Two genotypes that differed only by single allele differences at one or two loci with high rates of estimated null allele frequencies were considered to be the same individual. The set of genotypes that includes genotypes "corrected" for null allele dropout is referred to as the "allelic-dropout corrected dataset." Probability of identity ($P_{(ID)}$) per marker and similarity levels between genotypes were determined using GIMLET (Valiere, 2002).

Population estimates

To estimate total population size from genotypes of both spring and winter sampling sessions I used closed models in Program MARK (White & Burnham 1999; Pledger 2000). The low number of deer visiting the snares suggests that there was a behavioral response resulting in trap shyness, so models that included behavioral responses were considered among different closed models. Multiple models were run using a combination of closed, closed heterogeneity, and full closed heterogeneity models. The best model was chosen based on the lowest Akaike Information Criterion (AIC) value that differed from the AIC values from other models (ΔAIC) by at least a value of 2. I performed separate MARK population analyses for winter and spring sampling sessions.

Results

Hair Collection, Probability of Identity, and Null Alleles

I collected 387 samples during both sampling sessions. Of those, 139 contained at least five hairs for extraction and amplification (Wills 2008). Five microsatellite loci had sufficient power so that only 0.7% of full siblings would have the exact same genotypes (Valiere 2002). Unbiased estimate of $P_{(ID)}$ was 0.0000008 (Table 1); estimates for dropout-corrected genotypes were $P_{(ID)unbiased}$ = 0.0000004, $P_{(ID)sibs}$ = 0.007.

Table 1 Values of expected and observed heterozygosities, along with the probability of identity values for each locus ($P_{(ID)}$*unbiased*, and $P_{(ID)}$*sibs*).

Marker	$\mathbf{H_{Exp}/H_{Obs}}$	$\boldsymbol{P_{(ID)unbiased}}$	$\boldsymbol{P_{(ID)sibs}}$
BM6506	0.85/0.66	3.21E-02	3.33E-01
OhD	0.84/0.81	1.14E-03	1.13E-01
OhN	0.75/0.63	9.05E-05	4.48E-02
BM4107	0.75/0.72	8.37E-06	1.79E-02
RT24	0.73/0.54	8.24E-07	7.35E-03

After one round of PCR, 37 of the 139 samples composed of five or more hairs returned fully resolved genotypes across five loci. A second round of PCR was performed on loci of samples that failed to amplify at a given locus. After this second round of PCR there were 70 complete five-loci genotypes, and 67 of those 70 were unique. The sexing reactions identified 16 males, and 46 females. Five samples that returned a full five-locus genotype failed to yield sexing results. After analyzing these 67 distinct genotypes in Micro-Checker I determined that loci *OhN*, *RT24*, and *BM6506* exhibited significant frequencies (≥0.05) of null alleles (Table 2).

Table 2 Null allele frequencies for each of the five loci calculated in Micro-checker. The five columns associated with each locus show different statistical estimations of null allele frequencies, as in Van Oosterhout (2004) The three loci with significant frequencies of null alleles are noted with an "*".

Locus	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
OhD	0.0108	0.0168	0.0151	0.0151
*OhN**	0.0756	0.0845	0.0671	0.0671
*BM6506**	0.0896	0.1049	0.0877	0.0877
*RT24**	0.2004	0.2594	0.1609	0.1609
BM4107	0.0121	0.0120	0.0102	0.0102

I found that eight genotype pairs differed from one another by one allele, and 20 genotype pairs differed from one another by one allele at one or two loci. These problematic genotype pairs differed such that one genotype was homozygous for one allele while the other was a heterozygote where one allele of the heterozygote was the same as the allele in the homozygote). Based on the high probability that these slight differences between genotype pairs resulted from allelic dropout and did not represent truly novel genotypes, each pair was considered a single genotype that was "captured" and "recaptured." This set of genotypes with pairs that were collapsed based on presumed allelic dropout comprises the "allelic dropout corrected genotypes."

Table 3 Model selection results from mark-recapture estimates of white-tailed deer in Presque Isle Park, Marquette MI during winter.

Model	AICc	Δ AICc	Parameters	Deviance	$\hat{N}$	SE
Mo	37.12	0.00	2	28.88	45	22.02
Mb	39.22	2.09	3	28.87	50	94.94
Mh	39.22	2.10	3	28.88	45	22.02
Mtb	42.06	4.93	12	11.19	16	0.17*-003
Mt	43.33	6.21	9	19.65	42	20.09
Mbh	43.51	6.39	5	18.65	50	94.93
Mtb	44.64	7.51	10	18.61	17	3.92
Mth	66.08	28.96	18	19.65	42	20.09

Table 4 Rank of population models in MARK for white-tailed deer in Presque Isle Park, Marquette MI during spring.

Model	AICc	Δ AICc	Parameters	Deviance	$\hat{N}$	SE
Mbh	48.81	0.00	4	45.69	927	1751.50
Mb	52.16	3.35	3	51.07	55	17.19
Mo	52.44	3.63	2	53.38	179	82.57
Mh	56.50	7.68	4	53.38	179	82.57
Mth	60.43	11.62	18	28.11	854	1506.14
Mtbh	63.27	14.45	19	28.80	38	3.83
Mtb	63.57	14.75	17	33.38	38	3.64
Mt	64.07	15.25	16	36.01	173	79.33

Population Estimates

After correcting the genotypes for allelic dropout the number of distinct individuals decreased from 67 distinct individuals captured to 51. Two of these individual genotypes were identified twice within the same day. For winter, the closed model without time dependence, individual heterogeneity and behavioral response, (Mo) ranked highest as being supported by data

with an estimate of 45 (CI = 24 – 125) animals (Table 3). For spring, the model including individual heterogeneity in capture probability and behavioral response (Mbh) ranked highest.

Discussion

This was the first attempt to derive a population estimate for ungulates using non-invasive genetic sampling and capture-mark-recapture modeling (Ebert *et al.* 2010). The population estimate for the winter sampling period ($\hat{N}$ = 45: 95% CI = 24-125) that took place from 8 February–11 April 2008 was reasonably similar to the 40 animals counted on the deer drive performed on 29 March 2008. However, the population estimate for the spring sampling period ($\hat{N}$ = 55: 95% CI = 41-124) that took place from 11 May – 17 June 2008 was considerably higher than the 31 animals counted on the deer drive performed on 6 December 2008. Implausibly low standard error estimates suggested that my data were too sparse to support this model, and other models containing heterogeneity, and that its ranking was spurious. Thus, I selected the behavioral model (Mb) as the most appropriate for estimating population size ($\hat{N}$ = 55 CI = 41-124). In the rankings created by MARK (Mb) had a higher AIC value than (Mo), but the different in AIC values between Mb and Mo (ΔAIC) values suggest equal support for each. While there was equal support for both models, (Mb)'s estimate was more reasonable than (Mo)'s ($\hat{N}$ = 179 CI = 86-446). The low recapture probability (c = 0.013) shows that deer entering the snare once were unlikely to reenter the snare suggesting trap shyness as an issue. The estimates derived from the genotyping data are larger than the drive count data, but this would be expected because the genetic sampling occurred over winter and spring periods that lasted two months and one month long, respectively. The drive counts probably provide accurate counts of deer on the

peninsula on the day that they occur, but the hair snares were collecting samples from deer that were present on the island over the course of many days. In leaving hair snares set up for weeks or months, I was able to sample deer that presumably move in or out of the park via the narrow isthmus. In this case, non-invasive genetic sampling provided a mechanism to detect deer that used the park habitat but were not present in the park on the day of the drive counts.

Confidence Intervals

The broad confidence intervals for each estimate could be explained by sampling effort, genotyping errors, behavioral response, or simply sparse data. Otis *et al.* (1978) suggested CMR studies use four sampling sites for every home range. Presque Isle has a radius much smaller than the seasonal average home range of white-tailed deer (1.6 km: Smith 1991). It is possible that deer move through the residential and industrial barriers onto the mainland suggesting that Presque Isle is a small portion of larger home ranges used by individual deer (Fig. 5). Due to the (now illegal) supplemental feeding of deer in the park it is reasonable to believe that deer treat it as a seasonal territory with a stable food source. If we consider Presque Isle a territory for resident deer, then the six sampling sites was adequate per Otis *et al.*'s (1978) recommendation.

Fig. 5. Map of isthmus connecting Presque Isle Park to the mainland of Marquette County, MI. © Google Earth 2010

Genotyping errors can bias population estimates. As suggested previously, quality control measures should be taken to prevent misidentifying individuals (Paetkau 2003; McKelvey & Schwartz 2004). I addressed misidentification by "shadow effect" by determining the $P_{(ID)}$ for my

marker set (Valière *et al.* 2002). This ensured a low likelihood that distinct individuals would share identical genotypes ($P_{(ID)sibs} = 0.008$) leading to a negative bias in the CMR estimates. Null alleles that lead to "allelic dropout" necessarily create an inflated number of new individuals. Through an analysis of null allele frequencies, I was able to account for this positive bias by correcting genotype misidentifications attributable to allelic dropout. *Post hoc* analysis of allele frequencies showed three loci had excessive homozygosity, which suggests a high rate of allelic dropout (Van Oosterhout *et al.* 2004). Pairwise comparisons between all genotypes suggested that some "unique" genotypes only differed from other genotypes by one or two alleles. These genotypes were subsequently considered the same genotypes (and thus captures and recaptures), which diminishes the possibility of allelic dropout leading to positive bias in these CMR estimates.

We observed low recapture rates, possibly attributable to deer having little need for minerals at this time of year. Indeed the BuckJam© provided a scent (sweet apple) to initially draw deer to the lure, but the more persistent substance was a concentrated mineral salt not necessarily attractive to deer on Presque. To lure deer repeatedly to the snares, a more attractive food lure might have been preferable, but park regulations prevented supplemental feeding of deer. Bear studies with similar goals have successfully used food consumables as a lure, drawing animals to the snares for both captures and recaptures to a greater degree than this study (Boulanger *et al.* 2004; Triant *et al.* 2004).

I hypothesized behavioral model (Mb) would best describe data for winter and spring. This was true for spring, when Mb ranked highest in AICc analysis (Table 4). Winter data was best described by the simple closed model (Mo) which is often chosen in the case of sparse data (Lancia *et al.* 2005) (Table 4). It is reasonable to assume that trap shyness occurred in both

sessions, regardless of Mo ranking highest for winter. Similar bear studies have used heterogeneity models (Mh) to derive population estimates (Boulanger *et al.* 2004; Bellemain *et al.* 2005). This contrasts with my study in that behavioral response to baited snares, or passive fecal collection was not an issue.

Finally the sparseness of my data, specifically in the number of recaptures, probably contributed the most to the broad confidence intervals. For the spring (Mb) and (Mo) ranked equally in AIC. Lancia *et al.* (2005) suggests that (Mo) will rank high when the amount of data is limited. I justified using the estimates from the highest ranked (Mb) because the low recapture rates (c = 0.013) suggest deer became trap shy for some reason (i.e. unattractiveness of the lure). The sparseness of data created a strong positive bias in the (Mo) ($\hat{N}$ = 179 CI = 86-446) relative to the two drive counts bracketing this study while (Mb) accounted for low recapture rates when deriving population estimates. Ultimately a larger number of recaptures would have increased the precision of the MARK analysis.

Sex Ratios

A final component of this study was an investigation of the sex ratio of this deer population. The molecular genetic sexing data showed there is a female bias of 3.27 females for every male. Other work with a western Pennsylvanian population that was subjected to low harvest rates (like the Presque Isle Park population) showed a sex ratio of nearly 1:1 (Woolf & Harder 1979). The female-biased sex ratio in Presque Isle Park is perhaps attributable to the restricted geographic area of the park (a peninsula) and the social behavior of white-tailed deer. In white-tailed deer, females are more likely to remain part of matrilineal territories and later establish their own territories close by their natal territory (Kilpatrick & Spohr 2000). Males older

than one year of age are less likely to remain with the matrilineal groups and often disperse alone. Thus, I would expect to identify large numbers of resident females and few males in a small geographic area. In certain contexts social pressures from matriarchs force yearling bucks out into peripheral territory (Hawkins *et al.* 1971; Ozoga and Verme 1985) which could explain the low number of males detected. The pressure exerted by females could be compounded by the limited area of Presque Isle Park as males forced to disperse would have to traverse the residential and industrial barrier (Fig. 5). Once across the isthmus, without an equal pressure to return to the island, I would not predict males would cross this barrier from the mainland. The limited area of Presque Isle Park is also not ideal for males since they generally use larger home ranges than females (Gavin *et al.* 1984). Presque Isle Park is more than likely occupied by one or several matrilineal groups of related females and their associated yearling male fawns, and less-frequently visited by several transient adult male deer.

Conclusion

This study was the first to use a baited hair snare technique to non-invasively collect genetic samples from a wild population of ungulates. These samples were genotyped using five variable microsatellite loci, taking into account genotyping errors due to the dropout of null alleles. These genotypes were then used in a "capture-mark-recapture" analysis, where the first instance of a genotype appearing was considered its initial "capture and mark" and any repeated occurrence of that genotype was considered its recapture. The unattractiveness of the lure to deer probably reduced the likelihood of recapture and this broadened overall confidence intervals. My estimates of population size were larger than the drive counts of deer in the Park that occurred before and after my sampling periods. Drive counts only record deer present on the peninsula on the day of the drive count, whereas the hair snares sampled the deer in the Park for a period that

spanned five months. The longer time period for this study, and the ability of deer to move on and off the peninsula via the connecting isthmus could explain the larger genotype-derived estimates, as more deer were likely to have been both moving onto and off the island and be sampled and identified. Molecular sex-identification of hair samples revealed a 3.27:1 female bias sex ratio in the Presque Isle population. The residential and industrial barriers of Presque Isle could encourage the movement of males off the peninsula while discouraging other males from entering from the mainland. Ultimately the use of non-invasively collected genetic material proved useful in analyzing both population size and sex ratio in this study.

CHAPTER 3: GENETIC STRUCTURING IN ISLAND POPULATIONS OF WHITE-TAILED DEER

INTRODUCTION

Heterogeneous distribution of individuals of wildlife species can create genetically subdivided populations. These subdivided populations consist of subpopulations which are interbreeding groups of individuals exchanging genes and groups of subpopulations comprise a metapopulation. The genetic connectedness between subpopulations will theoretically depend on the geographic distance between subpopulations and on the amount of migration between them (Barton & Slatkin 1986). Smaller subpopulations separated by longer distances have the potential for a high degree of genetic differentiation (Madsen *et al.* 1996; Saccheri *et al.* 1998; Eldridge *et al.* 1999). The amount of gene flow between subpopulations is a product of raw geographic barriers and distance (MacArthur & Wilson 1967).

Populations of white-tailed deer (*Odocoileus virginianus*) can be divided into smaller subpopulations separated by geographic and behavioral barriers (Donnelly & Townson 2000; Gerlach & Musolf 2000; Pálsson 2000). Behavior contributes to genetic structure among these subpopulations, but generally geographic barriers create a much more influential obstacle to genetic migration (Mathews & Porter 1993). While vagility of white-tailed deer can reduce differentiation between subpopulations, extreme spatial separation has been shown to create genetic differentiation (Purdue *et al.* 2000; Miller *et al.*, 2010). This study focuses on the influence of water as a geographic barrier to geneflow in white-tailed deer rather than distance alone.

Genetic Structure in Metapopulations

Metapopulation genetic structure can range from a situation where all subpopulations have the same allele and genotype frequencies ("panmixia") to a situation where each subpopulation is unique in its allelic and genotypic composition. The degree of similarity between subpopulations can be measured in several ways. First as a measure of genetic connectedness among subpopulations, the observed heterozygosities of subpopulations can be compared to the expected global heterozygosity of the metapopulation (F_{ST}) (Mills & Allendorf 1996; Balloux & Goudet 2002; Conner & Hartl 2004). F_{ST} measures can then be used to estimate genetic migration of individuals per generation (*Nm*) between populations (Barton & Slatkin, 1986). Population differentiation can also be identified by comparing allele frequencies within subpopulations to allele frequencies of the total population (Weir & Cockerham, 1984; Bohonak, 1999; Balloux & Goudet, 2002).

In extreme cases of geographic isolation (i.e., large bodies of water, or mountain ranges) deer populations could potentially show subpopulation genetic structure that is best described by an island biogeographic model. In an island biogeographic model, genetic differentiation between populations on the island and the mainland is proportional to the distance of an island from the mainland (MacArthur & Wilson 1967; LeCorre & Kremer 1998; Conner & Hartl 2004; Abdelkrim *et al.* 2005). Furthermore, once colonized, larger islands (which presumably support larger populations) should be able to maintain higher levels of the original genetic variation that was present in the source mainland population (Conner & Hartl 2004). This study evaluated these two predictions from island biogeographic theory using white-tailed deer populations on two islands of Lake Superior. Oak Island and Grand Island have different geographic characteristics

which could contribute to the genetic differentiation of white-tailed deer populations on each. Given the relatively longer distance from the mainland and smaller size, I predicted that the deer population on Oak Island will have higher inbreeding statistics,

Fig. 6 Map of Oak Island and its relative proximity to the Bayfield Peninsula of Wisconsin. © Google Earth, 2010

fewer alleles and a lower number of generational migrants to the mainland when compared to the population of the larger Grand Island which is closer to the mainland. A third study site, Presque Isle Park, is a large island-like land mass connected to the mainland by a thin isthmus of land (see chapters 1 and 2 for more details). Since Presque Isle Park is directly connected to the mainland, I predicted that it should show the lowest measures of isolation (inbreeding, rare alleles, migration) of all three study sites.

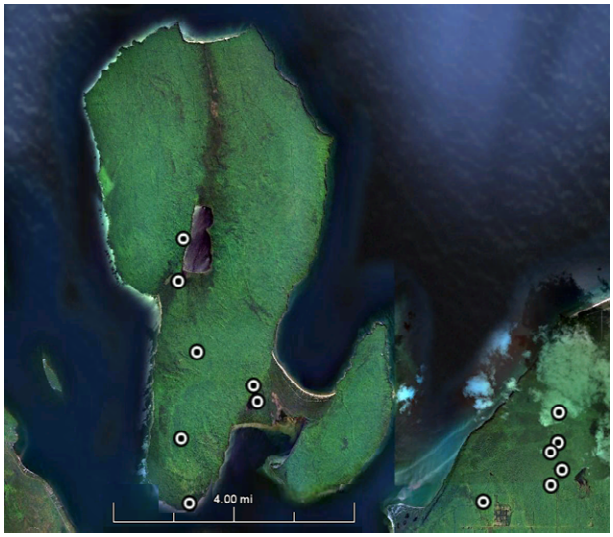

Fig. 7 Map of the locations of snares installed on Grand Island and in PRNL. © Google Earth, 2010

Materials and Methods

Study Sites

Oak Island has a total land area of 21km^2 and is located 2.12km from the nearest mainland of Bayfield County, Wisconsin (Fig. 6). The forest over story is dominated by mixed hardwood and hemlock forests consisting of red oak, eastern hemlock, balsam fir, sugar maple, and yellow birch (Taber 1990). White-tailed deer genetic samples (and genotypes) were available from Oak Island (46º56'13.75"N, 90º43'41.67"W) and the adjacent Bayfield Peninsula of Wisconsin (46º48'11.80"N, 90º45'46.70"W), through collection by another study (Belant, *unpub. data*) that used harvested individuals and hair snares. Grand Island (46º29'13.68"N, 86º40'06.70"W) is a 58-km^2 island located 0.63km off the the nearest mainland shore of Pictured Rocks National Lakeshore in Michigan's upper peninsula (46º31'04.21"N, 86º24'32.44"W) (Fig. 7) (Silbernagel *et al.* 1998). Pictured Rocks National Lakeshore (PRNL) is composed of deciduous forests and spruce (*Picea* spp.), tamarack (*Laryx laricina*) and Northern white-cedar (*Thuja* occidentalis) wetlands (Metzger & Schultz 1981). Study sites used for sampling deer were located in habitat that can support deer during the spring. Seven genetic sampling sites were set up on Grand Island and six sites were set up on the adjacent mainland of PRNL (Fig 7). Sites were chosen no less than 15m away from road or footpath. Presque Isle Park of Marquette County, Michigan is a peninsula that terminates in a large, ovate land mass jutting into Lake Superior, connected to the mainland by a thin strip of land (Fig. 5). Presque Isle Park snare locations were chosen to be separated by no less than 300m.

Installing Snares

Using fencing staples, 15.5 gauge four-pronged barbed wire was attached to 3-4 trees about 70 cm above ground creating a polygon (Belant *et al.* 2007). In areas of uneven ground, soil was added or removed to maintain a consistent 70 cm height of the barbed wire. One liter of Buckjam© (Evolved Habitats) was poured over a small pile of sticks in the center of the

enclosure, and it was refreshed at regular two-week intervals. Bright yellow information signs were stapled to each anchor tree facing out from the snare.

Tissue Collection and DNA Extraction

Weekly sampling from Grand Island and Pictured Rocks took place from May 24th 2008 to July 3rd 2008 while daily sampling on Presque Isle Park took place from February 9th 2008 to June 17th 2008. At Presque Isle Park, I collected hair samples every 2-3 days during winter (8 February-19 April) and daily during spring (12 May-17 June) periods. I removed hair from barbs using flame-sterilized forceps and deposited hair into individually labeled 1.5mL microcentrifuge tubes. Date, snare number, and barb number were recorded for each sample. To prevent contamination, I used a butane-lighter to destroy any remnant tissue on barbs. Collected samples were stored at 4˚C until DNA extraction using DNEasy Tissue Kits® (Qiagen) according to manufacturer's directions. DNA was suspended in the AE buffer and stored at -20°C until genetic analysis was performed.

Microsatellite Amplification and Genotype Scoring

Mirosatellites were amplified and genotypes were scored using the same techniques as are outlined in Chapter 2. All Oak Island and Bayfield Wisconsin genotypes included the same five loci used to genotype Grand Island, PRNL, and Presque Isle samples.

Population Genetic Analyses

I used FSTAT v. 2.9.3 (Goudet, 2001) to estimate F_{ST} values among subpopulations, under the stepwise mutation model that assumes each locus is selectively equivalent and that alleles can mutate to another allelic class. This program assigns weights to alleles to accommodate for sample size variation and uses bootstrap replications to obtain confidence

intervals for each locus across the entire population (Weir & Cockerham, 1984). I also used FSTAT to calculate inbreeding statistics (F_{IS}) at the subpopulation level and assumed all populations descended separately from a single ancestral population that was in Hardy-Weinberg and linkage equilibrium. I used GENEPOP 4.0 (Rousset, 2007) to estimate *Nm* by the private alleles method (Barton & Slatkin 1985), also under the assumption that each subpopulation had reached equilibrium between genetic drift and immigration.

Results

Hair Collection

I collected 61, 123, and 383 hair samples from Grand Island, PRNL and Presque Isle Park, respectively. Eleven hair samples were collected from the seven snares installed around Marquette County, Michigan, but none returned adequate genotypes for analysis. All samples successfully amplified at four to five loci were included in population genetic analysis, resulting in 11, 21, and 69 genotypes from Grand Island, PRNL, and Presque Isle Park, respectively. Additionally, 47 genotypes from Bayfield County and four genotypes from Oak Island were included in this analysis.

Table 4 Population differentiation parameters calculated for the two island-mainland population pairs and for all populations (including Presque Isle Park) compared to one another.

Population	F_{ST}	**95% CI**	**SE**
Oak Island/ Bayfield Peninsula	-0.0170	-0.027- -0.007	0.006
Grand Island/ Pictured Rocks	0.0347	0.022-0.048	0.006
All Populations	0.1040	0.065-0.146	0.018

Population Genetic Statistics

Neither of the island populations showed significant differentiation from their mainland counterparts. However, of the island-mainland pairs, Grand Island and PRNL were more similar (*Fst* = -0.0170) than Oak Island/Bayfield Peninsula (*Fst* = 0.0347) (Table 4). Moderate differentiation was observed between all populations (F_{ST} = 0.1040). Intrapopulation inbreeding statistics (F_{IS}) were determined on a per subpopulation basis to see if any region displayed excess homozygosity. All three Michigan subpopulations showed significant levels of inbreeding (F_{IS} > 0.05), and Grand Island showed the highest level of inbreeding (F_{IS} = 0.220) of the three Michigan subpopulations examined here. Oak Island and the Bayfield Peninsula shared 2.1 migrants per generation, while Grand Island and Pictured Rocks shared 1.2 migrants per generation (Table 6). There were 2.6 genetic migrants per generation shared between all subpopulations.

Table 5 Inbreeding statistics for each of the different subpopulations.

Subpopulation	F_{IS}
Oak Island, WI	-0.071
Bayfield Peninsula, WI	-0.043
Grand Island, MI	0.220
Pictured Rocks, MI	0.153
Presque Isle, MI	0.160

Table 6 Number of migrants shared by subpopulations per generation as calculated in GENEPOP 4.0 (Rousset 2007).

Population	***Nm***
Oak Island/Bayfield Peninsula	2.136
Grand Island/Pictured Rocks	1.222
All Populations	2.559

Discussion

Differentiation per Subpopulation

The population F_{ST} values for both of the Lake Superior island-mainland pairs examined in this study are low enough to doubt any significant level of differentiation (F_{ST} <0.05). While differentiation between populations was not significant the varying levels are worth considering in relation to each of their respective island-mainland population relationships. These data suggest that the degree of difference in the distance to the mainland does not result in a distinct pattern in differentiation (F_{ST}). If distance were the dominant factor shaping genetic structure, Oak Island should have showed the greatest degree of differentiation from its mainland counterpart, but it did not. There was no significant differentiation between Oak Island and Bayfield County subpopulations (F_{ST} = -0.0170). All alleles of Oak Island genotypes occurred at least once in the mainland population of Bayfield, Wisconsin. Grand Island and PRNL are twice as close to one another as Oak Island and Bayfield County are to one another. Grand Island-PRNL populations showed a higher level of genetic differentiation than the Wisconsin populations although both were still non-significant.

Apart from distance to the mainland, the size of the island and time since colonization of deer also suggest that Oak Island should have shown a higher degree of differentiation. The force of genetic drift is stronger in smaller populations, so larger islands (like Grand Island compared to the smaller Oak Island) should sustain larger populations which would help maintain rare alleles. Larger islands should maintain higher levels of genetic variability and heterozygosity than smaller islands. The longer a population is isolated from its source population with limited migration the more likely it would be that unique alleles would be isolated in small populations

and move to fixation (Balloux & Goudet, 2002). Another simpler explanation for the lack of differentiation between populations could be that the water isn't a barrier preventing deer from moving between subpopulations. The capacity for deer to cross during ice over, or swim during warmer weather could negate any population differentiation for the island populations (Michael 1965).

Inbreeding in Subpopulations

The Oak Island (F_{IS} = -0.071) deer population showed non-significant levels of inbreeding, while Grand Island population showed the highest levels of inbreeding (F_{IS} = 0.220) (Table 5). In both cases the island exhibited higher inbreeding coefficients than the mainland counterpart (Table 5). Given the smaller population sizes and limited dispersal distances, islands are more prone to encouraging non-random mating among relatives, which leads to high inbreeding statistics (Conner & Hartl, 2004). Presque Isle Park showed moderate levels of inbreeding (F_{IS} = 0.160) even though it is connected to its mainland counterpart by a narrow isthmus (Table 5). The population of deer in Presque Isle Park has experienced two bottlenecks which could contribute to high inbreeding. The first bottleneck occurred as a result of the release of deer from the Presque Isle Park Zoo which led to few founding deer contributing to the local deer genepool in the park (J. Bruggink personal comm.). The second bottleneck occurred in 2001 when the Presque Isle Park deer herd was culled from 100 to 15 deer. Either of these coupled with a relatively insulated population

Fig. 8 Map of Apostle Island National Lakeshore (Bayfield County, WI) with Oak Island circled. © Google Earth, 2010

with little immigration from the mainland, would serve to increase the inbreeding coefficients on this island (Abdelkrim *et al.* 2005).

Migrants between Subpopulations

The low estimates of *Nm* along with the low F_{ST} values could be indicative of low population sizes for each of the islands. Since *Nm* is a measure of the proportion of migrants contributing to the gene pool, two deer migrating to Oak Island would reduce differentiation to zero if only 20 deer inhabited the island (Conner & Hartl 2004). In some cases one migrant per generation can drastically reduce F_{ST} values regardless of the population size (Mills & Allendorf 1996). When compared to the low F_{ST} values the lack of differentiation could also be due to a recent expansion to these islands, implying that these subpopulations are the result of relatively recent emigrations from the mainland. If subpopulations are divided, a low proportion of migrants per generation can reestablish panmixia. Perhaps more significantly, low levels of migration (m>0.05) can maintain genetic homogeneity between subpopulations that were once continuous (Conner & Hartl 2004). Estimates of migration rates for Oak Island were nearly twice those of Grand Island (Table 6). Oak Island is exposed to the mainland at its southwest shoreline, while the rest of its contiguous shoreline is separated by six other islands belonging to the archipelago by an average distance of 2.94km (*min* = 2.19km, *max* = 3.94) (Fig. 8). Oak Island could exhibit reduced differentiation as result of migration from the mainland to Oak Island but also as a result of emigration and immigration to and from the other six peripheral islands. While none of the six islands immediately surrounding Oak Island has permanent populations of deer, there are records of temporary populations on some of these islands. In contrast, Grand Island only receives migrants from its mainland counterpart, PRNL. During the winter Oak Island and Grand Island generally each experience complete ice cover of the surrounding waters of Lake Superior

(NOAA, Great Lakes Environmental Research Laboratory, Ann Arbor, MI), which could conceivably provide ice bridges for deer to cross between islands and mainland. Migration across the winter ice bridges would still be affected by the total distance to the island which means the same relationship between distance and *Nm* would apply.

The proportion of migrants per generation among all subpopulations was high relative to migrants per generation exhibited between the two island/mainland scenarios (Table 6). This is a result of using the private alleles mathematical model to determine *Nm* (Barton & Slatkin 1986). In expanding a dataset to include more samples there is the increased chance of capturing individuals from different subpopulations with a shared rare allele at a locus. The more broadly samples are collected from a metapopulation, the more likely it is that rare alleles will be shared among individuals.

Conclusion

Neither of the island populations of white-tailed deer examined in this study showed significant levels of differentiation from their corresponding mainland population. However, Grand Island showed higher levels of inbreeding than did the smaller Oak Island. Although Oak Island is further from the Wisconsin mainland than Grand Island is from PRNL, it is likely that some geographic characteristics in the Wisconsin system – specifically the number of surrounding islands – creates a “stepping-stone” effect for migration and this lowers the inbreeding levels on the island. Oak Island is farther from the mainland than Grand Island is, but there are more island “stepping stones” connecting it to the mainland. Oak Island may also itself be a stepping-stone island for deer that move between the mainland and the more peripheral islands of the Apostle Islands archipelago. If Oak Island is a stepping stone then it would experience a higher amount of migration to and from its shores and thus lower its inbreeding

coefficient. Identifying long term trends of inbreeding and population differentiation for not only Oak Island but also the other source island populations could clarify the complete migration picture of the archipelago. The only source for immigrants to Grand Island is the mainland (there is no comparable island archipelago. The higher level of inbreeding on Grand Island relative to Oak Island supports the possibility that there simply are too few immigrants maintaining heterozygosity in this population. It is also possible that the low levels of differentiation for all locations are a result of ineffective geographic barriers failing to prevent migration of deer within these systems. It is certainly possible that deer swim from island to mainland (or reverse) during the spring and summer and cross the ice during the winter, maintaining genetic panmixia. A long term genetic study could potentially reveal differences between mutli-island archipelagoes versus single island populations.

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APPENDIX A

Institutional Animal Care and Use Committee Approval

Northern Michigan University

Continuing Education & Sponsored Programs
1401 Presque Isle Avenue
Marquette, MI 49855-5325

MEMORANDUM

January 11, 2008

TO: Dr. Alec R. Lindsay
Department of Biology

Jerrold L. Belant
Co Principal Investigator

FROM: Cynthia A. Prosen, Ph.D.
Dean of Graduate Studies & Research

CP

RE: **Application to use Vertebrate Animals**

Application # IACUC 076
Approval Period: 5/1/2007-5/1/2009

The Institutional Animal Care and Use Committee have approved your application to use vertebrate animals in research, "Assessing non-invasive sampling techniques and conservation genetic models with white-tailed deer (*Odocoileus virginianus*)".

If you have any questions, please contact me.

kjm

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