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Two Fields, One Pellet: Combining Demographics and Population Genetics Through Non-invasive Sampling of Snowshoe Hare Fecal Pellets in Michigan.

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TWO FIELDS, ONE PELLET: COMBINING DEMOGRAPHICS AND POPULATION

GENETICS THROUGH NON-INVASIVE SAMPLING OF SNOWSHOE HARE FECAL

PELLETS IN MICHIGAN

by

Genelle Uhrig

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ABSTRACT

TWO FIELDS, ONE PELLET: COMBINING DEMOGRAPHICS AND POPULATION
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The University of Wisconsin-Milwaukee, 2019
Under the Supervision of Dr. Emily Latch

As climate continues to change at a rapid rate, species are increasingly vulnerable to the

resulting environmental changes. This is especially true for species whose fitness is closely

linked to climate-associated environmental conditions. One of these vulnerable species is

snowshoe hare, *Lepus americanus*, who depends on the timing and duration of snowfall to

provide camouflage when they go through seasonal pelage changes from brown in the summer to

white in the winter. Whereas snowshoe hare are stable across the core of their range, populations

along the southern range edge are experiencing declines due to climate driven environmental

changes that cause a mismatch between pelage color and the background environment (e.g.,

white hare pelage against a brown snowless background), making hare more conspicuous to

predators, reducing survival and leading to localized extirpations. My thesis aimed to gather

baseline demographic estimates (e.g., density) and to characterize fine-scale patterns of genetic

diversity and gene flow of snowshoe hare subpopulations in a portion of their southern range

within the Hiawatha National Forest-East (HNFE) in Michigan. I combined the two fields of

demography and population genetics through non-invasive genetic tagging, in which snowshoe

hare fecal pellets (n=847) representing 160 individuals were used in both spatially explicit

capture-recapture and genetic analyses. Snowshoe hare density varied across occupied sites

(range=0.02-0.838 hares/ha) and was low overall (>1 hare/ha), but similar to other areas along their southern range edge. Density was positively correlated with horizontal vegetation cover at 50 cm ($p=0.007$) and 100 cm ($p=0.01$), and conifer stem density ($p<<0.001$), habitat features previously found to promote snowshoe hare density. Genetic diversity estimates of heterozygosity and allelic richness were high and similar across sites. I found 3 distinct genetic clusters indicating population structure, but this pattern is weak and genetic differentiation was low. Overall, these results indicate that despite low snowshoe hare population densities, genetic diversity remains high and genetic differentiation weak, contrary to expectations for declining populations. Significant differentiation observed between some sites suggests that these populations are beginning to become isolated and would benefit from management actions to increase connectivity between these sites. The variation we see in density across our sites is likely driven by heterogeneity in the landscape and in order to maintain the adaptive potential of snowshoe hare in the HNFE in the face of climate change, maintaining high densities of snowshoe hare populations and subsequent levels of genetic diversity and gene flow should remain a focus of forest managers. This thesis provides the first assessment of snowshoe hare genetic diversity, population genetics, and localized density in this region and supports the effectiveness of using non-invasive genetic tagging to monitor snowshoe hare populations along the southern edge of their range as they face increased vulnerability to climate change.

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LIST OF ABBREVIATIONS

ELT   Ecological Land Type

HNFE   Hiawatha National Forest- East

IFWD   Inland Fish and Wildlife Department

PCR   Polymerase Chain Reaction

SCR   Spatially explicit Capture-Recapture

SSH   Snowshoe hare

UP   Upper Peninsula

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my dreams, we have made it through and will be together again soon.

Introduction

Climate change is an integral component of species conservation planning. Environmental changes induced by climate alter species distributions by changing interactions between organisms and their environment (Foden et al. 2018, Scheffers et al. 2016). These changes may have positive or negative effects on populations, depending on the climate factors to which a species is adapted and the degree of adaptive plasticity (Foden et al. 2018). When environments change, individuals either adapt to new conditions, move to favorable environments, or perish. Gradual environmental changes afford more time for adaptation or dispersal. When changes in the environment are rapid, particularly when new conditions reduce fitness, populations that cannot adapt or disperse quickly enough to offset fitness consequences will decline or be lost entirely (Pease et al. 1989). Species most vulnerable to climate change include those whose fitness is closely linked to one or more climate-associated environmental conditions; whose adaptive capacity is low (e.g., poor dispersal ability, low genetic variation, long generation times, low reproductive output); and whose population sizes are small (Foden et al. 2013). These vulnerabilities occur throughout the living world as seen in polar bear declines due to loss of sea ice vital for resting and capturing prey (Rode et al. 2010), bleaching of coral reefs due to warming ocean temperatures that symbiont zooxanthellae cannot survive (Baker et al. 2008), and the extinction of Bramble Cay melomys due to habitat destruction from sea level rise (Gynther et al. 2016).

Even in species with globally stable populations, impacts of climate change can often be seen in widespread extirpations along range edges (Wiens 2016). Populations along the trailing edge of species shifting distributions (i.e., warm range boundaries) may already be living at the limit of their thermal or hydric tolerance, and thus may be more sensitive to climate-induced

environmental changes than populations in the core of the species range. For example, cold-adapted snowshoe hare (*Lepus americanus*) are abundant in northern boreal forests of Canada but are experiencing severe declines and extirpations along their southern range edge (Sultaire et al. 2016a, Diefenbach et al. 2016, Burt et al. 2016, VDGIF 2015 NatureServ 2019). These declines are closely tied to the environment; survival declines with decreased snow cover, through increased predation because their white winter pelage is conspicuous against an environmental background that is increasingly without snow (Zimova et al. 2016). This climate-change induced pelage-environment color mismatch causes high mortality in snowshoe hares and projected to cause continued population declines and local extinctions into the future (Zimova et al. 2016, Diefenbach et al. 2016). Other climate variables linked to localized extinctions of snowshoe hares include an increase in mean maximum temperature from 15 May to 19 Jan and a decrease in number of days with snow on the ground (Burt et al. 2016). Habitat loss and fragmentation also likely plays a role in localized declines, yet mounting evidence suggests that climate change is the main driver behind localized extinctions (Burt et al. 2016, Sultaire et al. 2016*a*, Diefenbach et al. 2016, Zimova et al. 2016). Since climate is changing at an alarming rate (NOAA 2019; USGCRP 2017, 2018), many species are unable to outpace climate change and conservation actions have become critically important for species persistence.

Accurate baseline data on species distribution and abundance informs conservation actions to mitigate the consequences of climate change and ensure the persistence of managed populations. This baseline demographic information, coupled with landscape, environmental, and community data, provide a clearer picture of species ecology and climate vulnerability. For example, another climate vulnerable lagomorph species, American pikas (*Ochotona princeps*), have experienced declines in the Rocky Mountain region and studies have utilized abundance

estimates in relation to landscape and climate variables to better understand their vulnerability (Yandow et al. 2015). Recent advancements in spatially explicit capture-recapture (SCR; Borchers and Efford 2008) have improved modeling of population demography and allowed for the scaling of estimates, provide insight into space usage and animal movement, and reduced bias of edge effects found with non-spatial capture-recapture methods (Borchers 2012). Capture-recapture approaches have been further enhanced by the utilization of noninvasive genetic techniques to 'capture' individuals without handling them, using materials they leave behind in the environment that contain their DNA (e.g., hair, scat, feathers). Noninvasive capture-recapture approaches can provide robust estimates of density, often with less field effort, permits, behavioral biases, and animal stress than live trapping (Cheng et al. 2017, Ferreria et al. 2018, Sabino-Marques et al. 2018).

The genetic information obtained in non-invasive genetic tagging studies can also provide insight into mechanisms driving population dynamics and help prioritize conservation efforts. High genetic variation is maintained in large, connected populations, but is eroded as populations decline and become more isolated. Small, isolated populations are not only less demographically stable than large populations, but also are more susceptible to further genetic diversity loss, inbreeding depression, and reduced ability to adapt to changing environments. As management occurs at more local scales, genetic variation estimates can also be used to assess gene flow and connectivity between subpopulations to understand local population dynamics and identify populations that would benefit from conservation and management efforts.

The objective of this study is to assess local snowshoe hare population dynamics with the following aims: 1) estimate density and capture probability of snowshoe hare populations using non-invasive genetic tagging with spatially explicit capture-recapture modeling, and 2)

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characterize fine-scale patterns of genetic diversity and gene flow across the landscape. These aims were investigated in Michigan, a state where snowshoe hare populations are experiencing localized declines—as many historically occupied sites are no longer occupied (Burt et al. 2016)—and are also vulnerable to climate change (Wonch et al. 2015, unpublished report), driving the need for continued research. We predict that density and genetic diversity will vary across the landscape in accordance with heterogeneous declines in localized snowshoe hare populations in the region, and that a lack of connectivity between remaining patches of suitable habitat has led to population differentiation. These aims will yield baseline data on snowshoe hare ecology to assist managers in understanding how snowshoe hare are distributed and connection of populations to guide management activities to mitigate the negative consequences of climate change.

Methods

Study Area

Our study was conducted in the East Unit of the Hiawatha National Forest (HNFE) in the Upper Peninsula (UP) of Michigan, a 1,604 km^2 area managed by the US Forest Service. The HNFE spans across Chippewa and Mackinac counties and bordered in the north by Lake Superior and in the south by Lakes Michigan and Huron (Fig. 1). Land cover types in highest proportion consist of white pine (*Pinus strobus*), red pine (*Pinus resinosa*), and jack pine (*Pinus banksiana*), secondary land cover types consist of maple, beech, and birch and to a lesser extent, aspen-birch (USDA 2006).

Site selection

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The Inland Fish and Wildlife Department of the Sault Ste. Marie Tribe of Chippewa Indians

(IFWD) selected sites in support of a multifaceted research project on snowshoe hare populations

in the HNFE. As such, sites were selected in a manner that would facilitate collection of a variety

of data (e.g., dynamics, disease assessment, and population genetics) using several methods.

Sites were randomly selected using a Generalized Random Tessellation Stratified design and

based on Ecological Land Type (ELT), a designation given by the US Forest Service in which

classifications differ in vegetation composition, soil type, and fire regime (USDA 2006). Three

sites were randomly selected for each of 6 ELT classifications (40/50/90, 10/20, 60, 30, 70, and

80; ELTs 70A and 70B, and 80A and 80B were combined) with a minimum area of 0.2 km^2, for

a total of 18 sampling sites. Site areas encompassed at least three snowshoe hare home ranges

(seasonal home range size 0.03-0.06 km^2; Feierabend and Kielland 2014) and ranged from 0.207

km^2 to 0.785 km^2 (Table 1).

Field methods

   Pellet collection.—During the winters of 2016-2017 (Year 1) and 2017-2018 (Year 2),

the eighteen sites were sampled by IFWD technicians. Nine sites were sampled in Year 1 and

nine sites in Year 2 (Fig. 1). Sampling occurred during the winter to minimize pellet (and thus

DNA) degradation, to facilitate pellet discovery, and to ensure population closure as hares do not

typically disperse during winter (though home range sizes may increase—see Feierabend and

Kielland 2014). Pellets were collected, on average, 2 days after snowfall to eliminate the need to

age the pellet and avoid collecting older pellets now covered by snow.

   Sites were sampled using a partially unstructured search-encounter method in which each

site was surveyed on at least 2 and up to 4 occasions. Each occasion consisted of a unique

transect that was determined *a priori* to cover as much of the site area as possible. Predetermined transects were loaded onto GPS units and used as general guides, but technicians were able to deviate from the transects to follow tracks if general proximity to the predetermined transects were maintained (therefore the design is considered partially unstructured). GPS units tracked technicians' movements and these track log transects were used in density modeling. Once a pellet was encountered, GPS coordinates were recorded, and the pellet was placed in a 5 mL tube with gloved hands. Because an individual hare can defecate more than 450 pellets per day (Hodges 2000), a minimum of 1 meter spacing between collected pellets was upheld and limits of 50 total pellets per transect and 100 total pellets per site were set. Number of transect-sampling occasions was determined by the number of pellets collected on each occasion, so sites with few to no pellets were surveyed up to 4 occasions whereas sites with many pellets were surveyed on only 2 occasions. Following transect completion, pellets were stored in a -20°C freezer until being shipped on ice to UWM and then stored in a -20°C freezer until DNA extraction.

       Vegetation measurements.—During the winter of 2017-2018, vegetation structure and composition were measured to help identify habitat characteristics that best predict snowshoe hare density at our sites. Horizontal cover, the leading predictor of snowshoe hare occupancy and survival (Litvaitis et al. 1985, Holbrook et al. 2017, Sultaire et al. 2016b), was measured at 50 cm and 100 cm above snow level. Overhead (vertical) cover, number of conifer stems, and number of deciduous stems were also measured. Within each of our eighteen sampling sites, the above measurements were taken at 10 X-Y coordinates that corresponded to the location of live traps used in a separate study. For each X-Y coordinate, 4 random bearings were selected (*a priori*) and the presence or absence of overhead cover and both horizonal cover levels were

recorded at each bearing. Along the fourth bearing, the number of conifer stems, and deciduous

stems within a 4 m x 1 m transect were counted. Measurements at the 4 bearings were averaged

for the coordinate and then coordinates were averaged for the site overall, including stem

densities.

Lab methods

   *DNA Extraction, Amplification, and Genotyping—*Fecal pellets were extracted

using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) with modifications. For

steps 1-4, individual pellets were extracted in their 5 mL collection tubes to retain epithelial cells

that may have been removed during transportation. Additional modifications to the

manufacturers protocol included: 1.8µl of Inhibit EX Buffer added to the sample (Diefenbach et

al. 2016) followed by incubation in a shaker bath at 54°C for 20 minutes; prior to

homogenization, the outside of the pellet was washed with the buffer in the tube then macerated

with the pipet tip (Kovach et al. 2003) before vortexing for 2 minutes; to elute the DNA from the

column, 70µl of Buffer ATE was used, samples were incubated at 60°C for 5 minutes prior to

centrifuging, and a second elution was performed using the eluate. To prevent cross-

contamination, pellets were extracted in a dedicated low-quality DNA processing lab and gloves

were changed between each sample. Extraction negatives were implemented during each

extraction event and subsequently checked for contamination on ethidium bromide stained 1%

agarose gels and visualized under UV light. DNA extractions were quantified using a Qubit 2.0

Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), diluted to a standard concentration

of 5 ng/µl, and stored in a -20° C freezer.

A suite of 10 dimorphic microsatellite markers were optimized using 14-paired tissue and pellet samples from snowshoe hare trapped on several sites prior to the start of this study. All loci were developed for European rabbits and previously used in snowshoe hare studies (see Surridge et al. 1997, Burton et al. 2002, Schwartz et al. 2007, and Cheng et al. 2014) and included: Sat2, Sat3, Sat8, Sat12, Sat13, Sat16 (Mougel et al. 1997); SOL08, SOL03, SOL30 (Rico et al. 1994); and SOL33 (Surridge et al. 1997). Several markers were amplified in a multiplex (MP) including: SOL08 with Sat8 and SOL33 (MP1); Sat13 with SOL30 (MP2); and Sat16 with Sat12 (MP3). The remaining markers were amplified independently and subsequently co-loaded with one of the three multiplexes listed above for genotyping. Two markers, SOL03 and SOL30 (Rico et al. 1994), originate from the same locus and were amplified independently to verify genotypes and calculate error rates. SOL33 was dropped from analysis due to inconsistent sizing. A final suite of 8 markers was used for downstream analyses.

For microsatellite amplification, each 23 µL polymerase chain reaction (PCR) contained 15ng DNA, 1X ThermoPol buffer, 2mM dNTPs, 15µg bovine serum albumin (BSA), 0.27 – 0.54mM $MgCl_2$, 0.4 – 2.0 µM each forward and reverse primer, and 1 – 1.5 U Taq Polymerase in dH2O. PCR recipe and thermocycler conditions for each locus and multiplex are detailed in Supplementary Table S1. PCRs were diluted prior to genotyping at 1:10 for MP1 and MP2 and 1:5 for MP3, Sat3, and SOL3.

Samples were genotyped on an ABI 3730xl DNA Analyzer at the University of Wisconsin-Madison Biotechnology Center using Geneflo 625ROX size standard (Chimerx, Madison, WI, USA), and positive controls of known genotypes were included to ensure accurate scoring of alleles. Each plate genotyped contained a negative PCR control to identify cross-contamination from shipment or processing. Electropherograms were scored using GeneMarker

software (Soft Genetics, version 1.95). Genotyping errors can occur in all sample types but may be more common in genotypes generated from non-invasively collected samples, therefore, thorough re-amplification should occur to reach a consensus genotype and identify and eliminate errors. In this study, all pellets collected were genotyped at least once. We re-amplified all genotypes scored as homozygotes (to detect allelic dropout), a random subset (58%) of heterozygotes, low quality samples (peak intensity <1000 relative fluorescent units in GeneMarker), and any ambiguous genotypes. Ambiguity in multiplexed samples was resolved by running loci individually to minimize potential false alleles caused by nonspecific amplification of multiplexed loci. After initial genotyping, all rare alleles were re-amplified an additional two times. Genotypes were re-amplified up to four times to reach consensus. Overall error rate, and its components allelic dropout and false alleles, were calculated for each locus directly from the data. Additionally, each marker was assessed for errors stemming from null alleles, large allele dropout, and stuttering in software Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004).

Individual assignment and density modeling

Individuals were initially identified using the software COLONY version 2.0.6.4 (Jones and Wang 2010) using the clone method to identify and group matching genotypes (Wang 2016). A benefit of using this approach is it employs locus-specific error rates in determining clones (i.e. individuals). Parameters used included male and female monogamy with inbreeding and with clone; diploid and dioecious options selected; the Full-Likelihood method with long run time selected; no updating of allele frequencies; sibship scaling; and no sibship prior. Probability of identity (P_{ID}) and probability of identity for siblings (P_{IDsib}) were calculated in GenAlEx (version

6.503; Peakall and Smouse 2006, 2012) to assess the power of our microsatellite markers to identify individuals.

Density was estimated using spatially explicit capture-recapture (SCR) models in package '*secr*' version 3.1.8 (Efford 2018) in R version 3.5.1 within the RStudio interface which uses maximum likelihood to estimate the density of home-range centers (D) and capture probability (g0). In general, capture probability is a function of density and the spatial scale parameter σ, which represents the distance between home-range centers and the "trap" center (type of trap varies by collection method across studies) at which beyond this the capture probability declines. Model assumptions included a homogeneous Poisson point process for the density of home-range centers, a half normal detection function, and uniform probabilities of parameters across a site (i.e., null model: D~1, g0~1, sigma~1). Integration of the likelihood was achieved with a habitat mask that varied for each site, with buffer width of 4σ and spacing of 0.6σ (σ calculated with the RPSV function). Each survey transect differed in length and location within the site by occasion to cover as much area as possible, so usage was included in each model and was represented with a code of 1's (transect was used on occasion) and 0's (transect was not used on occasion). In a site with four transects, for example, the usage code for the first transect was 1000 whereas the usage code for the third transect was 0010.

Our sample collection method allowed us to test two trap-type methods available within *secr* as we searched each site (i.e. area) using transects, thus both the discretize method (used in area searches) and the transect method were appropriate options. Discretization is a process in which a transect or an area is split into effective "traps." First, model comparison of these two trap-type methods were conducted using two sites, one with a low number of recaptures and one with a high number of recaptures. Models for each method were evaluated using Akaike's

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Information Criterion correction for small sample size (AIC$_c$). The model with the lowest AIC$_c$ across methods for each of these sites was then used for all remaining sites. Additionally, models with discretizing of 1m, 5m, 10m, and 20m were compared to determine the optimal spacing of discretized transect "trap" segments.

In order to use the transect method within *secr*, transect lines were smoothed to reduce the number of vertices along a line to 200 (limit of package), followed by snapping pellet locations to the smoothed transect. This process was completed in QGIS version 3.8.1-Zanzibar (QGIS Development Team 2019). Since transect lines varied in length, the tolerance level (in meters) for smoothing varied for each transect. After smoothing, pellets were snapped to the nearest point on the new transect within a maximum of 10m.

For the initial model comparison of the two methods, the smoothed transects were discretized directly in *secr* to compare effects of discretization spacings of 1m, 5m, 10m, and 20m on parameter estimates and model fit. Of these, the best fit model had 20m discretization and was used for all sites in subsequent modeling. For the rest of the sites, discretizing directly in *secr* would have been a less efficient process (by having to smooth transects in QGIS first, then discretize), so transects were discretized into traps directly in QGIS and traps were then modeled as count detectors in *secr*. Original transects were transformed into traps by dividing them into 20m segments and the midpoint location of each 20m segment was used as the trap location. Since the transects varied in length, the number of traps per transect also varied with longer transects containing more traps and shorter transects containing fewer traps. Pellet points were then snapped to the nearest trap location within a maximum of 10m. Traps were modeled as multi-catch count detectors allowing for an individual to be captured in multiple traps and for multiple individuals to be captured in a single trap.

Parameter estimates were modeled by site, for sites with pellets, and in a multi-session model including all sites (those with and without pellets). As there were no individuals captured outside of their respective sample sites, independence was maintained allowing the use of a multi-session model. Utilizing a multi-session model and including sites that had no captures lends to a more realistic representation of parameter estimates for the whole study area as the parameter estimates are informed by both occupied and unoccupied sites. Precision of parameter estimates were determined as relative standard error (RSE=Standard Error of estimate/estimate; commonly CV), with precise estimates having RSE<20% (Pollock et al. 1990).

Population genetics analyses

Genetic variation was assessed for the total sample of individuals (n=158), for each year, and for sites with >5 individuals. We estimated genetic diversity using number of alleles per locus, allelic richness, observed and unbiased expected heterozygosity, and inbreeding coefficient F_{IS} in the R package *diveRsity* (version 1.9.90; Keenan et al. 2013). Significance was assessed with 1000 bootstrap resamples to obtain 95% confidence intervals (CIs) for allelic richness and F_{IS} and confirmed when CIs do not contain 0. Hardy-Weinberg Equilibrium (HWE) was assessed using exact tests in GenePop (version 4.6; Raymond and Rousset 1995, Rousset 2008) at 10,000 MCMC dememorizations, 500 batches, and 5,000 iterations per batch. Genotypic Linkage Disequilibrium (LD) was determined using FSTAT (version 2.9.4; Goudet 2003) at 5% nominal level and 10,000 permutations. Significance for both HWE and LD was assessed after implementing the Holm-Bonferroni correction for multiple tests (Holm 1979). Analysis of Molecular Variance (AMOVA) was conducted in GenAlEx (version 6.503; Peakall and Smouse 2006, 2012) to determine partitioning of genetic variation within and between sites.

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To quantify pairwise genetic differentiation between sites two complementary measures were estimated, $G_{ST(Nei)}$ and D_{Jost} using the R package *diveRsity* (version 1.9.90; Keenan et al. 2013). Significance was assessed with 10,000 bootstrap resamples to obtain bias corrected 95% CIs and indicated when CIs did not contain 0.

Patterns of genetic structure were characterized for populations with >5 samples (n=9) using complementary approaches in the software STRUCTURE (version 2.3.4; Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009) and R package *adegenet* (version 2.1.0; Jombart 2008). STRUCTURE was run independently 20 times, for each number of hypothesized clusters (K) from 1 to 12, using an MCMC burn-in of 500,000 steps and 750,000 repetitions. The parameter set with the maximum log likelihood included the admixture model with alleles correlated, the option of differing α by population, and using initial α=1/K (where K=number of sites) as described in Wang (2017). Structure Harvester (Earl and vonHoldt 2012) and Structure Selector (Li and Liu 2017) were used to determine the optimal number of clusters according to three methods: the mean log likelihood method (Ln P(X|K)) of Pritchard et al. (2000); the ΔK method of Evanno et al. (2005); and the estimators of Puechmaille (2016)—MedMeaK, MedMedK, MaxMedK, and MaxMeaK. Final structure plots were created in R using the individual output files from the software CLUMPP (Jakobsson and Rosenberg 2007), which aligned the outputs of membership coefficients from the 20 replicate runs. A Discriminant Analysis of Principal Components (DAPC) was conducted in the R package *adegenet* (Jombart2008). Because DAPCs are sensitive to the number of retained Principal Components (PCs), cross validation analyses were conducted to determine the optimal number of PCs to retain (highest mean successful assignment between training and validation subsets of the data)

and the lowest Root Mean Square Error (RMSE), conducted over a range of 10-50 PCs at 500 replicates each. The optimal number of PCs were then used in the final DAPC analysis.

Results

Non-invasive genetic tagging

A total of 847 fecal pellets were collected at 15 of 18 sites over the two sampling seasons (Year 1: n=269; Year 2: n=578). Number of pellets collected per site ranged from 0-100 (mean=47.05 pellets/site) with no pellets found at ELT 30 Sites 1, 2, or 3 (Table 1). All pellets were extracted, and quantified DNA concentrations ranged from <1 ng/µL to 111 ng/µL, with a mean concentration of 17.8 ng/µL. Overall amplification rate was high at 93.3%, with Year 1 amplification rate about 10% lower than Year 2 (86.7% and 96.6%, respectively). Consensus genotypes were reached for 824 pellets at the 8 loci, with the remaining pellets genotyped at 7 loci (n=14 pellets), 6 loci (n=6), 5 loci (n=2), and 1 locus (n=1). Pellets with missing data at more than 2 loci (n=3) were removed prior to individual identification.

All microsatellite markers were polymorphic and ranged from 9-27 alleles per locus (Table 2). Overall error rates per locus ranged from 0.4-3.5% (mean=2%), including dropout rate of 0.2-2.4% (mean=1.1%) and false allele rate of 0.2-1.4% (mean=0.5%). MicroChecker detected significant evidence of null alleles at locus SOL08 due to homozygote excess across allele size classes in sites 60-3 and 70-2. However, this locus was kept as the dropout rate was low (1%) and individuals at these sites contained on average 4.8 and 9 pellets each (i.e. replicates) which were amplified at least twice at this locus. Tests for deviation from Hardy-Weinberg equilibrium and genotypic linkage equilibrium were non-significant after sequential Bonferroni correction. The 8-microsatellite suite had high power (P_{ID}=6.9 x 10^{-11}; P_{IDsib}=4.19 x

10^{-4}; Table 2) identifying 160 individuals across the study area (Table 1; Year 1: n=54; Year 2: n=106). There was a positive correlation between number of pellets collected and number of individuals determined per site (r_{16}=0.903, p<<0.001).

<u>Demography</u>

Number of snowshoe hare recaptures across occasions within a site was low and ranged from 0-6 (Table 3; m_j Total for each site), with 8 out of 15 sites having no recaptures. By site, snowshoe hare density estimates ranged from 0.02 hares/hectare to 0.838 hares/hectare with RSEs of 21% to 81% (Fig. 2; Table 4). Sites with the fewest individuals had the highest RSE, indicating less precise estimates of density in small populations than in large populations. Snowshoe hare density for the entire study area, estimated from a multi-session null model, was 0.133 hares/hectare (RSE = 8.1%; Table 4). Site density was positively correlated with mean horizontal cover at 50 cm (r_{10}=0.728, p=0.007) and 100 cm (r_{10}=0.698, p=0.01), and conifer density (r_{10}=0.832, p<<0.001), characteristics associated with suitable hare habitat (Table 5). Correlations between site density and overhead cover and deciduous density were non-significant.

Detection function parameters varied across sites; capture probabilities (g0) ranged from 0.107 to 1.0 (multi-session model g0=0.49), and σ ranged from 45m to 140m (multi-session model σ=79.6m) (Table 4). Sites with low capture probabilities (<0.4) included those with a small number of individuals and few recaptures. Sites with high capture probabilities (>0.7) included those with a high number of individuals, yet some of these sites (60-3 and 80-3) had no recaptures across occasions. RSEs for capture probability and sigma estimates are generally

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below 20%, with the multi-session model having estimates at 9.8% and 3.3%, respectively

(Table 4). Overall, the multi-session model had the lowest RSE for each parameter estimate.

<u>Population genetics</u>

Genetic variation in snowshoe hare was high across the study area (Table 2). Heterozygosity and

allelic richness were high ($H_O = 0.73$, $A_r = 4.5$) and similar across sites (Table 6). An excess of

heterozygotes was detected at site 70-1 ($F_{IS} = -0.21$) but was likely due to small sample size

(n=5; Table 6). Genetic differentiation was low across the study area ($G_{ST} = 0.054$), with

statistically significant but weak population structure detected in 7 of 36 pairwise comparisons

(Table 7). Weak genetic structure among populations was also reflected in assignment tests,

where most individuals (63%) assigned to sites other than the ones in which they originated (Fig.

3). DAPC analysis generated a similar pattern, showing high similarity across sites and a lack of

pronounced genetic structure (Fig. 4).

Bayesian clustering analysis of the full dataset revealed 2-5 genetically distinct clusters

(Fig. 5). LnP(K) indicated 2 clusters; ΔK, MedMeaK, and MedMedK indicated 3 clusters; and

MaxMeaK and MaxMedK indicated 5 clusters (Fig. 5). These inferred clusters were only loosely

associated geographically. In the K=3 cluster solution, for example, sites 1020-3, 70-1, 70-3, 60-

3, and 80-2 were mostly assigned to cluster 1, sites 80-3 and 80-1 were mostly assigned to

cluster 2, and sites 405090-1 and 70-2 were assigned to cluster 3 (Fig. 5). Nearly all individuals

had a considerable portion assigned to each of the three clusters, indicating overall subtle

population genetic structure.

Discussion

Techniques for assessing populations are continually advancing wildlife management by expanding options available to achieve management goals. Our noninvasive mark recapture approach was effective for estimating density, detecting population structure, and quantifying genetic diversity of snowshoe hare populations in the UP of Michigan, providing the first density and genetic assessments for snowshoe hares in this region. As we predicted, density estimates varied across the landscape and were generally low. Weak genetic structure among populations suggests that either populations are connected by gene flow, or that isolation has not yet driven strong divergence. As populations in the UP continue to experience climate-associated declines, continued monitoring will be critical to understand dynamics of declining populations and to guide management activities. Techniques that provide a wealth of information per unit cost, such as the noninvasive capture mark recapture we used here, will continue to be important in monitoring snowshoe hare and other species affected by climate change.

In the UP, snowshoe hare density was low and similar to other southern populations. Across the HNFE, density was 0.13 hares/hectare, averaging 0.3 hares/hectare per site. Snowshoe hare densities in the southern portion of their range vary and typically peak at around 1-2 hares/hectare (Hodges 2000). In Yellowstone National Park, snowshoe hare densities estimated from high quantity pellet plots ranged from 0.31-0.84 hares/hectare (Hodges et al. 2009). In western Montana, winter snowshoe hare densities peaked at over 1.5 hares/hectare at one site, but most sites were at or below 0.5 hares/hectare (Griffin and Mills 2009). In northern Maine, snowshoe hare densities estimated from CMR were higher ranging 0.5-3.04 hares/hectare but were lower (0.15-1.5 hares/hectare) in other parts of Maine (Homyack et al. 2006). Lower densities in southern populations are expected as the ranges for cold-adapted species move

northward with warming temperatures. Ultimately, southern edge local extirpations are also predicted and have already been observed in the lower peninsula of Michigan (Burt et al. 2016). In the UP of Michigan, an eastward projecting peninsula bounded on the north by Lake Superior, the pattern of range shift might be different from a northward shift in contiguous habitats. To avoid extirpation, snowshoe hare would have to move toward the mainland before moving north impeding migration. Though the localized dynamics are likely complex, we might predict an overall east/west gradient of density as hare range shifts to the west. However, in our study there was no geographical trend in density (core/peripheral, north/south, or east/west). A lack of spatial pattern in density has been found in other studies (Hodges et al. 2009) and is likely driven by heterogeneity in the landscape. Thus, snowshoe hare population density in the UP might be driven primarily by the landscape. The HNFE is a mosaic of coniferous and deciduous forest stands and open areas, of suitable and unsuitable snowshoe hare habitat patches. Sites that provide suitable habitat are dense forest stands that provide a high percent of cover, both vertical and more importantly horizontal, while areas that are more open are less suitable (Lewis et al. 2011, Griffin and Mills 2009, Sultaire et al. 2016b, Thornton et al. 2013). Correlations between density and suitable habitat variables including horizontal cover and high conifer density measured at our sites align with these other studies. These findings are in line with existing habitat management recommendations to encourage snowshoe hare by promoting intermediate successional stage forests (Cook and Robeson 1945, Litvaitis et al. 1985, Monthey 1986, Koehler 1990).

Populations at low densities are more susceptible to loss of genetic diversity, increased inbreeding, and increased differentiation, especially when populations are isolated. Though our density estimates for snowshoe hare were low, we did not observe a corresponding reduction in

genetic diversity and increase in inbreeding. Snowshoe hare biology and behavior may act to maintain diversity despite low density through short generation time and high reproductive output (2-3 litters of 3-5 leverets each year; Aldous 1937, Stefan and Krebs 2001), a lack of sex-biased dispersal and philopatry (Burton and Krebs 2003), and a polygynandrous mating system, leading to a greater potential of exchange of genes within populations and gene flow between populations. We observed some genetic differentiation among populations, though the overall pattern was weak. This pattern of low differentiation among populations was also found in the core of snowshoe hare range (Burton et al. 2002), suggesting that weak genetic differentiation among populations might be a more universal pattern characteristic of snowshoe hare in general, where density and genetic differentiation are driven by local landscape heterogeneity.

Snowshoe hare persistence along the southern edge of their range will depend on their ability to adapt to warmer temperatures, less snowfall, and a shorter duration of snow during winter. While snowshoe hares along the southern edge of their range have shown little adaptability to the timing of pelage change (Zimova et al. 2014), winter pelage of snowshoe hares in Pennsylvania was less white and hair was shorter and less dense compared to snowshoe hares in Yukon, Canada (Gigliotti et al. 2017) where they experience much colder and snowier conditions during winter. In areas of the Pacific Northwest that experience little snowfall, some snowshoe hares remain in their summer brown pelage during winter (Mills et al. 2018), a trait that has been linked to introgression with jackrabbits that have brown pelage year-round (Jones et al. 2018). However, winter brown snowshoe hares have also been found in Pennsylvania (Gigliotti 2016), showing that adaptation for this trait may be more widespread. Adaptation to climate change likely will be critical to snowshoe hare persistence, whether at this trait or other traits directly or indirectly associated with climate. Adaptation arises from standing genetic

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variation, making the maintenance of overall genetic diversity in snowshoe hares a priority. As the climate changes, populations of snowshoe hare will continue to experience demographic decline along their southern edge. This pattern of loss is unlikely to occur in one continuous sweep northward, but more accurately described as localized extinctions throughout larger portions of their southern range (e.g., Burt et al. 2016). The heterogeneity of density and differentiation across our site supports this prediction, with no clear directional pattern of variation in density or diversity.

Non-invasive genetic tagging of snowshoe hare pellets is a technique used in several previous studies and was particularly successful in our study. In general, lower quality and quantity of DNA obtained using non-invasive methods typically yields lower amplification rates, a larger number of unusable samples, and high error rates compared to direct sampling methods (e.g., blood, tissue) (Taberlet and Luikart 1999, Ferreira et al. 2018, Beja-Pereira et al. 2009). These potential limitations were not realized in our study; we had high amplification rates which resulted in a high proportion of pellets used in our study and relatively low error. Other studies using DNA extracted from snowshoe hare pellets have had good, but varying, success depending on type of DNA being amplified (mtDNA vs. nuclear), sampling season, and age of pellets (Kovach et al. 2003, Schwartz et al. 2007, Cheng et al. 2017). In comparing degradation experiments conducted by Cheng et al. (2017) and Kovach et al. (2003), who sampled fecal pellets in summer and winter respectively, a clear advantage can be seen with collecting pellets in winter as amplification success remained high across weeks whereas summer pellet amplification success quickly declined after just a few days. Collecting pellets in winter likely slowed the degradation of DNA in our study as pellets were frozen upon deposition and remained frozen through collection until extraction. Summer pellet collection will be necessary

in many studies, for example to understand seasonal demographic parameters, but otherwise, collection of fecal pellets during colder temperatures can be advantageous to reduce or eliminate the challenges of degradation and aging that come with non-invasive genetic tagging.

Having high amplification success and low genotyping error led to high power to identify individuals from pellets, though the number of recaptures across occasions was low for our study and surprisingly so in sites with high numbers of individuals detected (e.g. sites 80-2, 80-3, and 60-3). The low number of recaptures was likely an effect of our sampling methodology. Our transects spanned beyond typical snowshoe hare home range size to capture as many individuals as possible and to cover as much of the site area as possible, resulting in few recaptures across occasions as each transect was unique and only surveyed once. This effect was exacerbated in areas with high numbers of pellets, where fewer transects and less total area were surveyed. Though recaptures across occasions were low, count (i.e. proximity) detector models in *secr* incorporate all detections (e.g. fecal pellets) of an individual (Efford et al. 2009, Efford 2011), so we were able to obtain relatively precise estimates of density and capture probability using this method (e.g. RSEs < 30%), especially when using all sites in a multi-session model (RSEs < 10%). Incorporating covariates such as trap level vegetation structure and forest stand type that capture heterogeneity in landscape variables relevant to snowshoe hare would likely improve precision of parameter estimates.

Management Implications

Snowshoe hares are a keystone prey species whose southern populations are expected to see continued declines due to climate change. Baseline demographic information for snowshoe hare populations can help managers target areas that would benefit most from conservation actions.

For example, habitat management for intermediate-age forests with high conifer density and horizontal cover would be expected to increase hare density and maintain connectivity among localized sites (Feldhamer et al. 2003). More robust snowshoe hare populations would increase their chances of survival in the face of climate change, by maintaining genetic variation important for species persistence and adaptation. Management activities that benefit snowshoe hare likewise help species that depend on healthy snowshoe hare populations. Continued monitoring of snowshoe hare populations will be important in an adaptive management framework for maintaining a healthy ecosystem.

Figure 1. Map of study area and sites where snowshoe hare pellets were collected during the winters of 2016-2017 and 2017-2018 in the Hiawatha National Forest-East in the Upper Peninsula of Michigan, USA. Names are listed next to sites and include the Ecological Land Type (ELT) identifier (first number) and site number for that ELT (second number). ELTs 70-A and 70-B and 80-A and 80-B were combined into 70 and 80, respectively, resulting in 6 ELT identifiers. Each of the 6 ELT identifiers have 3 sampled sites.
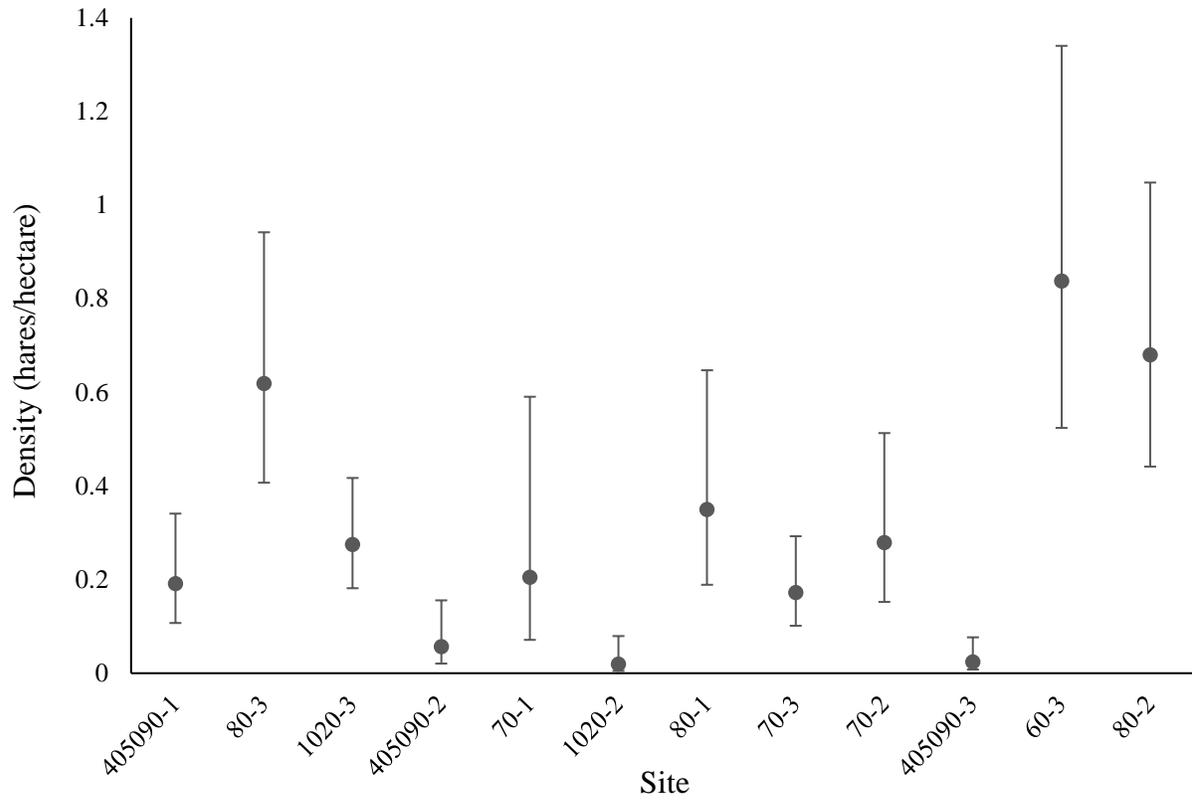
Figure 2. Snowshoe hare density estimates from genetic spatial mark-recapture by sites with greater than two individuals in the Hiawatha National Forest-East in the Upper Peninsula of Michigan, USA. Sites are arranged by latitude on the x-axis from left to right, going from North to South.
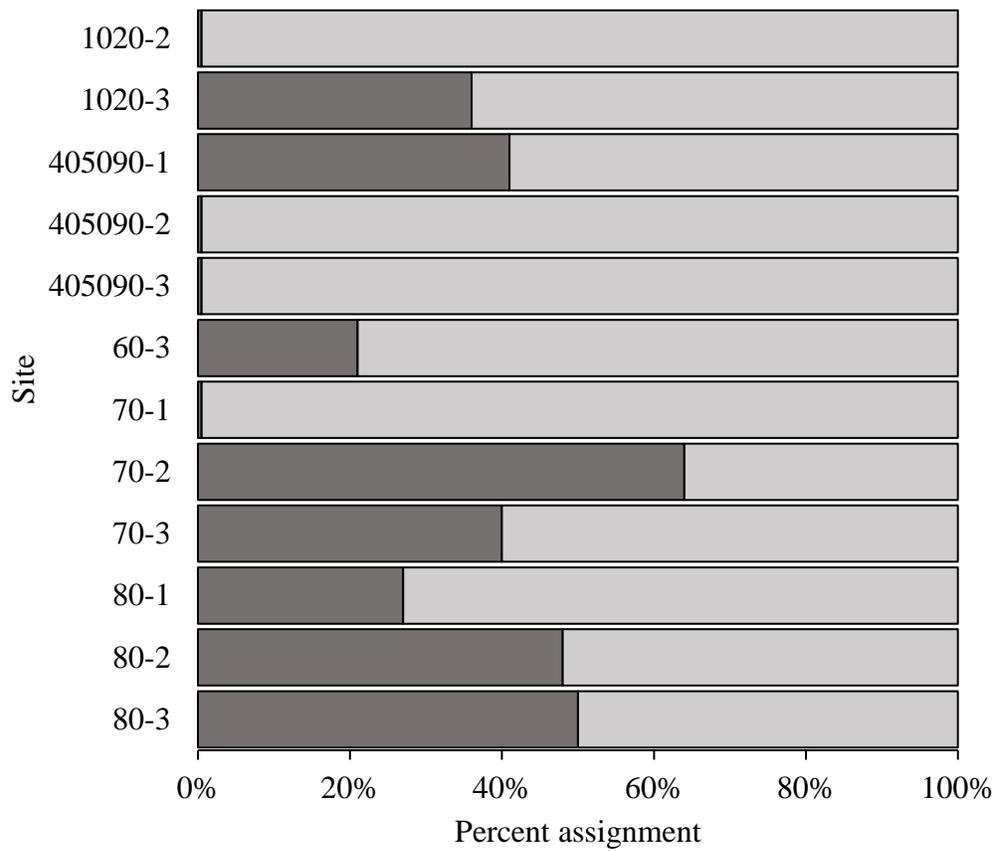
Figure 3. Percent of snowshoe hare individuals genetically assigned to their site of sample origin (dark gray) and to other sites (light gray), for sites in the Hiawatha National Forest-East, Michigan.
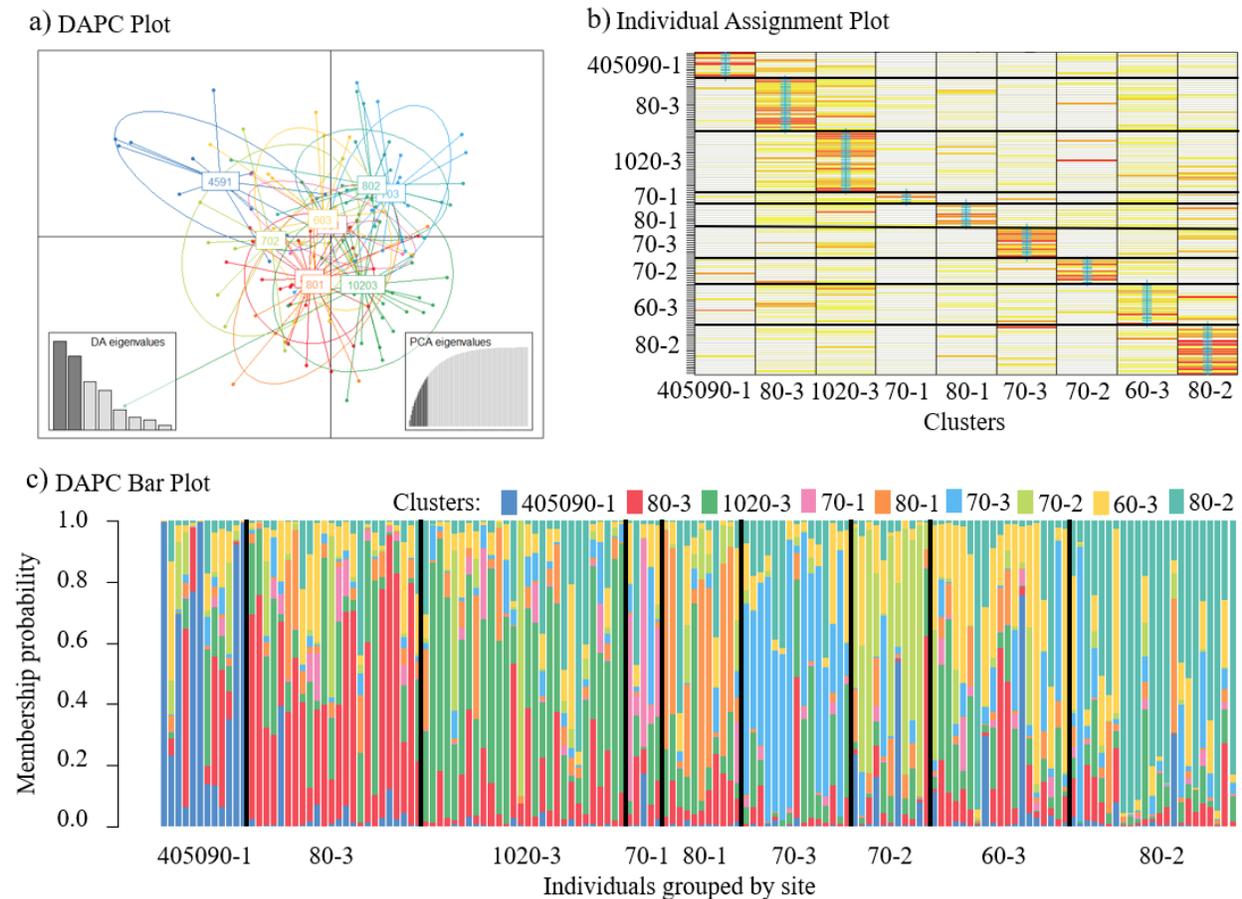
Figure 4. Discriminant Analysis of Principal Components (DAPC) of individual snowshoe hare multi-locus genotypes from the Hiawatha National Forest-East, Michigan, visualized as a) DAPC plot, b) individual assignment plot, and c) DAPC bar plot of individual membership proportions, to each K=site clusters. 17 principal components were retained with 62.4% variation conserved. The DAPC plot illustrates the variation of the first 2 eigenvalues as axis 1 and axis 2.
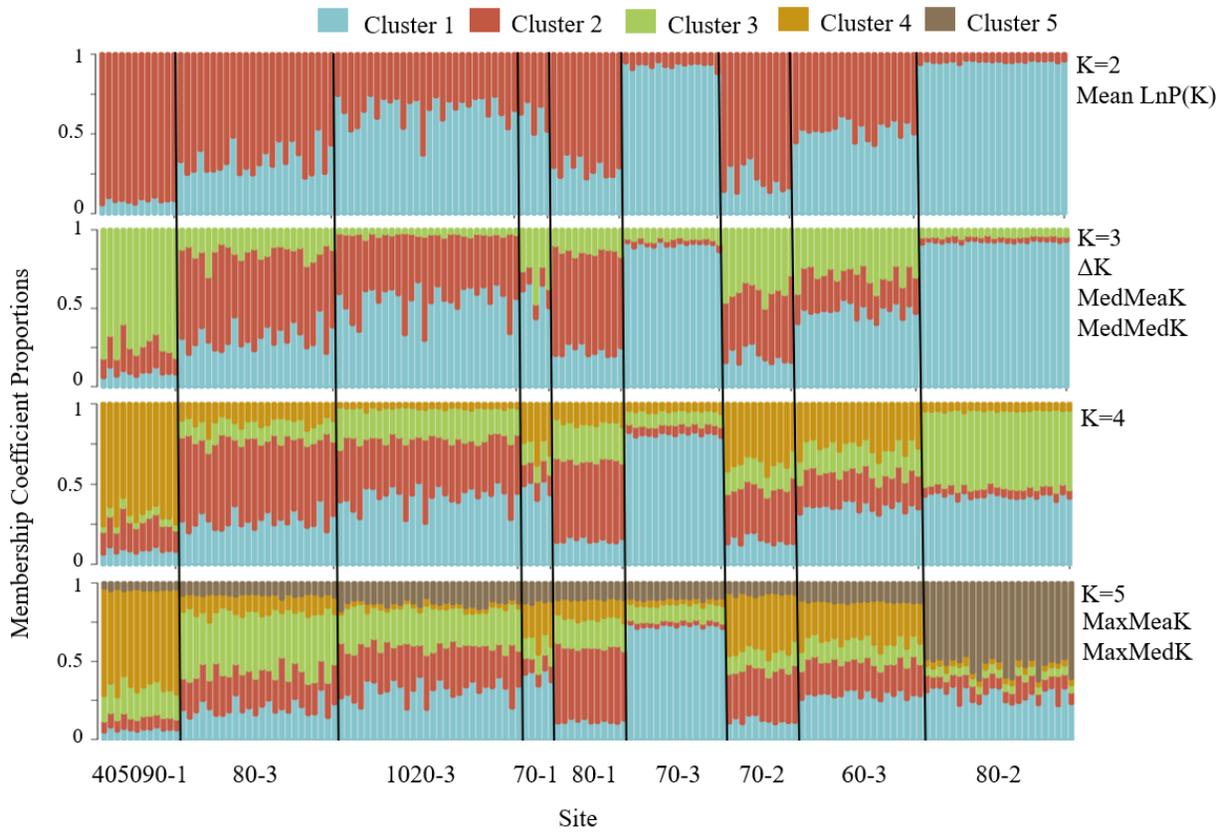
Figure 5. STRUCTURE plots of individual snowshoe hare membership proportions to clusters K=2 to K=5 and additional substructure of each site in the Hiawatha National Forest-East in Michigan. Bars represent individuals and are grouped by sites. The optimal K determined by common methods are listed next to that plot.

Table 1. Yearly sample collection summary of Ecological Land Type (ELT) and site visits, number of pellets collected, and number of individual snowshoe hares determined by genetic analysis of fecal pellet DNA in the Hiawatha National Forest-East in Michigan.

ELT	Site	Site Area (km^2)	Year Sampled	Transect	Date	Days since last visit	Pellets collected	Total pellets/ site	Number of individuals
30	1	0.466	1	1	1/6/2017	0	0	0	0
				2	2/16/2017	41	0		
				3	2/20/2017	4	0		
	2	0.304	1	1	2/2/2017	0	0	0	0
				2	2/17/2017	15	0		
				3	2/28/2017	11	0		
	3	0.742	2	1	1/30/2018	0	0	0	0
				2	2/2/2018	3	0		
				3	2/26/2018	24	0		
60	1	0.487	1	1	1/13/2017	0	1	1	1
				2	2/14/2017	32	0		
				3	2/20/2017	6	0		
	2	0.224	1	1	1/12/2017	0	7	7	1
				2	2/15/2017	34	0		
				3	2/27/2017	12	0		
	3	0.415	2	1	1/25/2018	0	50	100	19
				2	1/26/2018	1	50		
70	1	0.213	1	1	1/31/2017	0	12	12	5
				2	2/15/2017	15	0		
				3	2/27/2017	12	0		
	2	0.219	2	1	2/5/2018	0	50	100	11
				2	2/19/2018	14	50		
	3	0.649	2	1	1/17/2018	0	50	100	15
				2	1/18/2018	1	50		
80	1	0.216	1	1	1/16/2017	0	42	92	11
				2	1/17/2017	1	50		
	2	0.425	1	1	1/18/2017	0	32	96	23
				2	1/19/2017	1	64		
	3	0.778	2	1	12/13/2017	0	50	100	24
				2	12/13/2017	0	50		
10/20	1	0.207	1	1	1/30/2017	0	1	1	1
				2	2/16/2017	17	0		
				3	3/2/2017	14	0		
	2	0.785	2	1	1/10/2018	0	0	14	2
				2	1/16/2018	6	0		
				3	1/22/2018	6	14		

				4	2/13/2018	22	0		
	3	0.785	2	1	2/8/2018	0	50	100	28
				2	2/21/2018	13	50		
	1	0.616	1	1	1/5/2017	0	14	60	12
				2	2/9/2017	35	9		
				3	2/13/2017	4	37		
40/50/90	2	0.418	2	2	1/24/2018	0	0	28	4
				1	2/1/2018	8	0		
				3	2/14/2018	13	10		
				4	3/9/2018	23	18		
	3	0.785	2	1	2/6/2018	0	19	36	3
				2	2/15/2018	9	10		
				3	2/28/2018	13	0		
				4	3/2/2018	2	7		
Totals	36			99		412	847		160

Table 2. Locus diversity measures including all snowshoe hare individuals identified (n=160) over two winters (2016-2017 and 2017-2018) in the Hiawatha National Forest-East in Michigan.

Locus	Range (bp)	PID	PID_{sib}	N	A	A_r	H_O	H_E	F_{IS}
Sat8	95-152	0.015	0.300	160	27	25.5 (23 - 27)	0.91	0.91	-0.005 (-0.052 - 0.043)
Sat2	215-253	0.012	0.293	160	20	18.8 (17 - 20)	0.88	0.92	0.048 (-0.004 - 0.101)
Sat13	116-158	0.105	0.415	160	16	15.2 (14 - 16)	0.68	0.72	0.057 (-0.030 - 0.133)
SOL08	94-126	0.022	0.312	160	17	16 (14 - 17)	0.73	0.89	0.184 (0.100 - 0.260)
Sat16	89-115	0.032	0.324	159	13	12 (10 - 13)	0.79	0.87	0.087 (0.017 - 0.150)
Sat12	105-150	0.067	0.368	159	11	10.2 (9 - 11)	0.81	0.80	-0.009 (-0.081 - 0.056)
SOL30	165-185	0.291	0.556	160	10	8.4 (6 - 10)	0.56	0.54	-0.041 (-0.170 - 0.084)
Sat3	135-159	0.273	0.558	160	9	8.1 (7 - 9)	0.44	0.52	0.161 (0.031 - 0.290)
		$Total_{PID}$ 6.9E-11	$Total_{PIDsib}$ 4.19E-4	Mean	15.4	14.3 (14 - 15)	0.72	0.77	0.061 (0.025 - 0.089)

PID=probability of identity; PID_{sib}= probability of identity for siblings; N= number of individuals; A= number of alleles; A_r= allelic richness (LCL - UCL); H_O= Observed Heterozygosity; H_E= Expected Heterozygosity; F_{IS}=Inbreeding coefficient (LCL - UCL); LCL=Lower Confidence Limit; UCL=Upper Confidence Limit

Table 3. Capture summary of snowshoe hare in the Hiawatha National Forest-East, Michigan, by site and occasion (j). Capture summary for winter 2016-2017 is above double line while winter 2017-2018 is below. Detections refer to the number of snowshoe hare fecal pellets collected using an area-search method. Detectors are effective traps created by discretizing search transects into 20 m sections. Total m_j is the total number of individuals recaptured and Total M_j is number of individuals detected.

Site	j	nj	mj	uj	Mj	fj	Detections	Detectors	Det Visited
60-1	1	1	0	1	0	1	1	142	1
	2	0	0	0	1	0	0	118	0
	3	0	0	0	1	0	0	97	0
	Total	1	0	1	$M_4=1$	1	1	357	1
60-2	1	1	0	1	0	1	7	97	7
	2	0	0	0	1	0	0	87	0
	3	0	0	0	1	0	0	64	0
	Total	1	0	1	$M_4=1$	1	7	248	7
60-3	1	8	0	8	0	19	50	48	20
	2	11	0	11	8	0	50	34	25
	Total	19	0	19	$M_3=19$	19	100	82	45
70-1	1	5	0	5	0	5	12	127	10
	2	0	0	0	5	0	0	68	0
	3	0	0	0	5	0	0	62	0
	Total	5	0	5	$M_4=5$	5	12	257	10
70-2	1	9	0	9	0	5	50	73	22
	2	8	6	2	9	6	50	58	31
	Total	17	6	11	$M_3=11$	11	100	131	53
70-3	1	9	0	9	0	12	50	46	29
	2	9	3	6	9	3	50	85	32
	Total	18	3	15	$M_3=15$	15	100	131	61
80-1	1	7	0	7	0	7	42	35	23
	2	8	4	4	7	4	50	47	19
	Total	15	4	11	$M_3=11$	11	92	82	42
80-2	1	9	0	9	0	23	32	147	21
	2	14	0	14	9	0	64	147	41
	Total	23	0	23	$M_3=23$	23	96	294	62
80-3	1	14	0	14	0	24	50	71	24
	2	10	0	10	14	0	50	62	20

	Total	24	0	24	$M_3=24$	24	100	133	44
1020-1	1	1	0	1	0	1	1	99	1
	2	0	0	0	1	0	0	77	0
	3	0	0	0	1	0	0	59	0
	Total	1	0	1	$M_4=1$	1	1	235	1
1020-2	1	0	0	0	0	2	0	116	0
	2	0	0	0	0	0	0	106	0
	3	2	0	2	0	0	14	115	8
	4	0	0	0	2	0	0	116	0
	Total	2	0	2	$M_5=2$	2	14	453	8
1020-3	1	19	0	19	0	23	50	35	25
	2	14	5	9	19	5	50	67	22
	Total	33	5	28	$M_3=28$	28	100	102	47
405090-1	1	4	0	4	0	8	14	122	11
	2	2	2	0	4	2	9	26	8
	3	12	4	8	4	2	37	97	26
	Total	18	6	12	$M_4=12$	12	60	245	45
405090-2	1	0	0	0	0	3	0	132	0
	2	0	0	0	0	1	0	105	0
	3	2	0	2	0	0	10	119	5
	4	3	1	2	2	0	18	124	13
	Total	5	1	4	$M_5=4$	4	28	480	18
405090-3	1	2	0	2	0	1	19	171	9
	2	3	2	1	2	0	10	144	8
	3	0	0	0	3	2	0	111	0
	4	2	2	0	3	0	7	125	3
	Total	7	4	3	$M_5=3$	3	36	551	20

j=occasion; n_j=number of animals captured on jth capture occasion; m_j=number of marked animals captured on jth capture occasion; u_j=number of unmarked animals captured on jth capture occasion; M_j=number of distinct animals captured before the jth capture occasion; k=number of capture occasions; M_{jk}=Total number of distinct animals captured at site; f_j=number of animals captured exactly j times

Table 4. Spatially explicit capture-recapture parameter estimates of snowshoe hare populations in the Hiawatha National Forest-East, Michigan. Parameters include density of snowshoe hare activity centers (D; units=hares/hectare), snowshoe hare capture probability (g0), and inflection point of the half-normal detection function (σ; units=m) denoting distance between activity center and trap location (i.e. index of home-range size). Precision of each parameter estimate is determined by Relative Standard Error (RSE; RSE=Standard Error of parameter estimate/parameter estimate). Mean estimates of the site parameters are given, and parameter estimates for a multi-session model which included all surveyed sites. Parameter estimates are from the uniform null model: D~1, g0~1, and σ~1.

Site	D	D RSE	g0	g0 RSE	σ	σ RSE
405090-1	0.191	0.302	0.538	0.184	57.5	0.080
80-3	0.619	0.217	0.883	0.121	45.4	0.066
1020-3	0.275	0.215	0.333	0.235	139	0.090
405090-2	0.057	0.552	0.107	0.285	98.9	0.145
70-1	0.205	0.581	0.132	0.514	46.4	0.314
1020-2	0.020	0.818	0.534	0.353	64.8	0.227
80-1	0.350	0.322	0.785	0.112	70.1	0.086
70-3	0.172	0.275	0.566	0.166	116	0.074
70-2	0.280	0.317	0.584	0.124	78.2	0.072
405090-3	0.025	0.634	0.399	0.191	85.9	0.115
60-3	0.838	0.243	1.000	1.83E-18	45.4	0.070
80-2	0.680	0.216	0.406	0.157	45.3	0.084
Mean	0.309	0.391	0.522	0.154	74.5	0.113
Multi-Session	0.133	0.081	0.491	0.098	79.6	0.033

Table 5. Mean vegetation measurements at sites sampled for snowshoe hare fecal pellets in the Hiawatha National Forest-East, Michigan. Measurements include mean overhead cover (OHC), mean horizontal cover at 50 cm (HC50), mean horizontal cover at 100 cm (HC100), mean conifer tree density (ConD), and mean deciduous tree density (DecD).

Site	OHC	HC50	HC100	ConD	DecD
30-1	0.93	0.40	0.38	0.00	0.35
30-2	0.95	0.68	0.58	0.10	1.05
30-3	0.90	0.40	0.43	0.18	0.33
60-1	0.98	0.55	0.53	0.08	0.23
60-2	0.80	0.68	0.63	0.43	0.33
60-3	0.80	0.93	0.85	0.73	1.28
70-1	0.78	0.38	0.30	0.13	0.10
70-2	0.80	0.75	0.60	0.10	0.88
70-3	0.70	0.85	0.88	0.13	1.95
80-1	0.85	0.78	0.63	0.50	0.53
80-2	0.65	0.75	0.68	0.98	1.65
80-3	0.78	0.83	0.90	1.30	0.20
1020-1	0.48	0.40	0.53	0.33	0.85
1020-2	0.50	0.33	0.33	0.10	0.35
1020-3	0.58	0.53	0.65	0.60	0.00
405090-1	0.88	0.50	0.40	0.03	0.98
405090-2	0.85	0.60	0.53	0.13	0.55
405090-3	0.90	0.23	0.18	0.05	0.28

Table 6. Genetic diversity measures of snowshoe hare by sites with ≥5 snowshoe hares detected

in the Hiawatha National Forest-East, Michigan.

Site	N	A	A_r	H_O	H_E	F_{IS}	A_P
405090_1	12	6.25	4.1 (3.13-4.88)	0.72	0.71	-0.014 (-0.182 - 0.059)	0
80_3	24	9.63	4.9 (3.75-5.75)	0.74	0.75	0.005 (-0.082 - 0.047)	4
1020_3	28	10.00	4.8 (3.75-5.63)	0.69	0.75	0.086 (-0.010 - 0.152)	3
70_1	5	5.50	4.2 (2.75-5.5)	0.85	0.70	-0.210 (-0.551 - -0.191)	0
80_1	11	6.88	4.2 (3-5.25)	0.65	0.71	0.084 (-0.138 - 0.172)	1
70_3	15	9.00	4.7 (3.5-5.88)	0.72	0.73	0.014 (-0.151 - 0.092)	8
70_2	11	6.13	4.0 (3-5)	0.69	0.70	0.016 (-0.185 - 0.112)	1
60_3	19	8.75	4.6 (3.63-5.5)	0.74	0.73	-0.006 (-0.113 - 0.039)	1
80_2	23	9.63	4.7 (3.75-5.63)	0.74	0.74	0.006 (-0.111 - 0.077)	6
Grand Mean	16.4	7.97	4.5	0.73	0.73	-0.0017	24

N= number of individuals; A= number of alleles; A_r= allelic richness (LCL-UCL); H_O= Observed Heterozygosity; H_E= Expected Heterozygosity; $_UH_E$=Unbiased Expected Heterozygosity; F_{IS}=Inbreeding coefficient (LCL-UCL); A_P= private alleles: LCL= Lower Confidence Limit; UCL=Upper Confidence Limit.

Table 7. Pairwise population estimates of fixation ($G_{ST(Nei)}$) above diagonal and differentiation (D_{Jost}) below diagonal, of snowshoe hare populations in the Hiawatha National Forest-East, Michigan. Significant estimates are in bold.

Site	405090-1	80-3	1020-3	70-1	80-1	70-3	70-2	60-3	80-2
405090-1	…	0.015	**0.022**	0.006	**0.027**	**0.022**	0.011	0.011	**0.025**
80-3	0.057	…	0.002	0.003	0.007	**0.014**	0.013	0.007	**0.014**
1020-3	**0.113**	0.003	…	0.003	0.004	0.006	0.011	0.004	**0.010**
70-1	0.008	0.005	0.003	…	0.018	0.007	0.012	<0.001	0.012
80-1	**0.146**	0.018	0.013	0.035	…	0.018	0.004	0.005	0.011
70-3	**0.096**	**0.091**	0.017	0.013	0.057	…	0.015	0.007	0.011
70-2	0.045	0.053	0.040	<0.001	0.008	0.068	…	0.006	**0.018**
60-3	0.044	0.027	0.012	<0.001	0.021	0.014	0.026	…	0.007
80-2	**0.082**	**0.077**	**0.051**	0.003	0.053	0.058	0.043	0.015	…

Table S1. Polymerase Chain Reaction (PCR) components and thermocycler conditions for amplifying snowshoe hare DNA. Each reaction contains 3 µL of DNA (up to 15 ng total) and 20 µL of Mastermix.

Multiplex	Locus	Label	MgCl$_2$ (mM)	Primer (µM)	Denature at 94ºC (sec)	Anneal (temp ºC, sec)	Extension at 72ºC (sec)	Total # cycles
1	SOL8	6-Fam	0.54	0.22	60	63-60**, 60	90	36
1	Sat8	Hex	0.54	0.17	60	63-60**, 60	90	36
2	SOL30	6-Fam	0.54	0.43	60	60, 60	90	37
2	Sat13	Hex	0.54	0.43	60	60, 60	90	37
*	Sat2	Hex	0.54	0.57	60	56, 60	90	45
3	Sat12	Hex	0.54	0.39	30	60, 30	60	35
3	Sat16	6-Fam	0.54	0.43	30	60, 30	60	35
*	Sat3	6-Fam	0.27	0.43	30	66, 30	60	40

* Sat2 and Sat3 were amplified individually and co-loaded with multiplex 2 (Sat2) and multiplex 3 (Sat3)

** 3 cycles at 63ºC, -1ºC per cycle, then 33 cycles at 60ºC

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