# Assessing Population Estimation Protocols for Sitka Black-tailed Deer Using DNA from Fecal Pellets

Karin R. McCoy, Grey W. Pendleton, and Rodney W. Flynn



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## Abstract

Because previous approaches to index population fluctuations using pellet-group counts are imprecise and do not meet current deer management needs, we explored the use of fecal DNA for broad-scale population monitoring of Sitka black-tailed deer (Odocoileus hemionus sitkensis) in Region 1, Southeast Alaska. Before developing a standardized monitoring method, we needed more information to inform our decisions on sampling design, sample processing, and data analysis. We evaluated a "node and spoke" sampling design that we hoped would be more costeffective and require less-restrictive assumptions for estimating deer abundance and density over broad spatial scales and at varied deer densities. We also documented how frequently deer fecal pellets occur in different habitat types and in locations with different deer densities. Finally, we tested the efficacy of two different DNA sampling methodologies, evaluated the use of a suite of microsatellite markers in different study areas, explored the use of traditional versus spatial mark-recapture models for population estimation, and provided a population estimate for a watershed of management concern. We found that our initial suite of markers did not work for the lower genetic variation encountered in northern Southeast Alaska, so we developed a revised set that worked across several study areas. While our "node and spoke" sampling design yielded good DNA estimates with coefficients of variation (CV) less than 21%, it did not provide an advantage over other designs for density estimation. The design required closely-spaced transect clusters or clusters covering a larger area than we used; and was logistically inefficient. We recommend other configurations that could increase sampling efficiency, such as circular, box, triangular, or grid transecting. We recommend using a clustered survey design with a high density of transects in each cluster, especially in low-density areas. Our modeling indicated that spatial mark-recapture models were more robust than more traditional mark-recapture techniques to changes in sampling intensity. Although our SMR modeling yielded similar density estimates for a single-visit versus multiple visit sampling scheme, we caution single-visit approaches likely would not work well in areas with lower deer densities, due to lower fresh pellet encounter rates and recaptures. Pellet groups were found in all habitat types with high variability in encounter rates, even among patches of the same type. We found that neither of the DNA sampling methods we tested (surface swabbing and dry storage versus whole-pellet storage in ethanol) was better than the other, but that a combined use of both methods can yield an approximate 40% increase in genotyping success.

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# Introduction

Since the mid-1980s, the Alaska Department of Fish and Game (ADF&G), Division of Wildlife Conservation (DWC) has monitored trends in Sitka black-tailed deer (Odocoileus hemionus sitkensis) (SBTD) populations in Southeast Alaska using a systematic survey of fecal-pellet groups (PGs) (Kirchhoff and Pitcher 1988, McCoy 2011). Counts of PGs are made along straight-line transects ideally located within winter range of deer from sea level to 460 m (1,500 ft) elevation (Kirchhoff and Pitcher 1988); we will refer to this approach as straight-line transect sampling. Transects have been established throughout the region in old-growth forested watersheds with surveys conducted during spring to estimate activity of deer during winter. Under ideal conditions, straight-line transect sampling yields an unbiased estimate of PG density in the sampled habitat (e.g., old-growth forest) within specific watersheds, which can be converted to a deer population estimate by multiplying by the average deer defecation rate (Kirchhoff and Pitcher 1988). PG counts are confounded, however, by seasonal and weatherrelated variability that influences persistence of pellets in the environment, defecation rates, and detectability of pellets at different elevations and within different habitat categories (Kirchhoff and Pitcher 1988). Moreover, deer activity within winter ranges is strongly influenced by winter weather and snow conditions. Although there are ways to account for some of these sources of variation, the data collection required is labor intensive and increases costs and so is seldom done. Therefore, data from straight-line transect sampling in Southeast Alaska has historically contained considerable variation that is not related to abundance or densities of deer. All of these factors make it difficult to get precise, accurate population size, density, or trend estimates directly from PG count data (Brinkman et al. 2013).

An alternative method to straight-line transect sampling has been developed by Brinkman et al. (2010a) and is referred as path sampling. Path sampling protocols were designed to efficiently locate and sample PGs deposited by deer, extract and sequence DNA from those pellets, and use the resulting genotypes to estimate deer abundance (Brinkman et al. 2011). Path sampling involves following a compass bearing until a deer trail (i.e., path) is encountered, after which deer trails most closely following the bearing are followed (see Brinkman et al. 2010a, 2011 for further details on field protocols). Path sampling, unlike straight-line transect sampling, does not provide samples independent of deer movements even under ideal conditions. Therefore, path sampling will not provide unbiased estimates of PG density for the entire study area and cannot be used to estimate deer density via defecation rates. Path sampling would provide an unbiased estimate of change in deer abundance only if the paths sampled contain a constant proportion of the PGs in the study area across time, which usually will not be known. However, it is more efficient for encountering and collecting PG for DNA identification (Brinkman et al. 2013). Brinkman et al. (2010b) tested several DNA protocols suitable for extracting and amplifying DNA from fecal pellets, and identified a suite of polymorphic loci useful for distinguishing individual deer. They also developed a PG sampling design and procedures that maximized sampling efficiency and simultaneously minimized the degrading effects of wet weather on the epithelial-cell DNA adhering to pellets. They used mark-recapture analyses (Williams et al. 2001) to estimate population size using DNA-identified deer (Brinkman et al. 2011).

During 2010–2012, DWC staff, in conjunction with the U. S. Forest Service (USFS), conducted research to continue to develop the approach of Brinkman et al. (2010a, 2011, 2013). On Prince of Wales Island, Brinkman et al. (2011, 2013) calculated deer numbers by using a DNA-based, mark-recapture approach. In Brinkman (2011), they estimated deer populations in three

watersheds on Prince of Wales Island using Huggins closed models (Huggins 1991) in Program MARK (White and Burnham 1999, White 2008). They used detection locations to calculate the mean maximum distance between detections of individual deer (MMDM), which they then used as a strip boundary around each transect to estimate the effective sampling area. However, basing the effective sampling area on MMDM often leads to positive bias in density estimates (Ivan et al. 2013). Also, in Brinkman et al. (2013), they converted estimated deer numbers by transect to a linear density (i.e., deer/km of trail) by dividing the population estimate by the length of the surveyed paths (or straight-line transects in some cases).

In this study, we were interested in extensions to these methods, including new statistical models (e.g., Efford 2012) that would be potentially more cost-effective and estimate deer abundance and density over broad spatial scales and at varied deer densities. We also were interested in improvements in DNA techniques including choice of DNA microsatellite markers and improved collection and sampling methods. On NE Chichagof Island, we wanted to provide managers with an estimate of population density for the Pavlof watershed because of management concerns in that area. In Game Management Unit (GMU) 3, we wanted to obtain PG encounter rates by habitat category to assess the numbers of PGs we could expect finding in a low-density deer population.

# Objectives

Our objectives were: (1) conduct DNA analyses to develop a set of DNA markers that would be suitable for use in multiple study areas across the region, regardless of the genetic variation present, (2) compare the efficacy of two different methods of collecting and storing deer fecal DNA, (3) compare fresh and over-winter PG encounter rates among various habitat types in an area with low deer density, (4) compare PG encounter rates on path versus straight-line transects, (5) estimate and compare the number of deer in the Pavlof watershed on Chichagof Island in 2010–2011, and (6) evaluate a node-and-spoke sampling design and the effect of varying sampling intensities and deer numbers on population and density estimates.

# **Study Areas**

During 2010–2011, we sampled the Pavlof watershed on northeast Chichagof Island, located about 35 miles west of Juneau, Alaska (Fig. 1). Although deer abundance was believed to have declined recently, we expected the area to have moderate numbers of deer (>1.50 PG/20 m<sup>2</sup> plot based on straight-line PG transects in the Pavlof River watershed; McCoy 2011). In 2012, we conducted field activities in GMU 3 (Fig. 1) near Petersburg, an area expected to have very low deer abundance; PG/20-m<sup>2</sup> plot in Castle River (Kupreanof Island) has ranged from 0.1 to 0.5, with PG/20 m<sup>2</sup> plot in E Duncan (Kupreanof) and Woewodski (Mitkof) recently falling below 1.0 (McCoy 2011).



Figure 1. Location of study areas in Southeast Alaska. We conducted deer fecal DNA sampling during April–May in the Pavlof Study Area on NE Chichagof Island in 2010 and 2011, and the GMU 3 study area in 2012.

### Methods

#### FIELD SAMPLING, CHICHAGOF ISLAND: 2010–2011

In 2010 in the Pavlof study watershed (Fig. 1), we established 34 nodes (points) that represented proportional sampling of all habitat categories below 365 m elevation, and that were separated by at least 1200 m from other nodes (Fig. 2). From the center of each of these nodes, we laid out four 600 m transects with an initial orientation in each of the four cardinal directions resulting in a "node and spoke" design resembling a wagon-wheel, where each 'spoke' is a transect. This sampling intensity is roughly 3–4 times that of Brinkman et al. (2011). For each transect, we used the path sampling protocol described by Brinkman et al. (2011) of following deer trails to collect PG samples, with the exception that all transect segments within 30 m of the node center point followed cardinal directions in a straight line to avoid overlap where transects converge. We flagged each transect on the first survey to facilitate repeat visits.

Personnel consisted of 4–6 individuals, working in two-person crews (for safety). Each twoperson crew surveyed approximately two transects/day, usually requiring workdays of over 8 hours. Each transect was sampled twice more at 8–12 day intervals, for a total of 3 surveys per transect.

During the first survey period, we tried to only sample pellets judged to be from fresh PGs based on appearance. We graded all samples as good (clumped distribution with smooth surface, glossy sheen and/or layer of mucous) or average (intact pellets with smooth surface, but no clumped distribution, glossy sheen, or mucus) or poor (spread-out groups with rough surfaced pellets, often showing signs of decomposition) quality based on their appearance. We collected all good PGs, recorded a waypoint for all PGs encountered, and then removed all PGs from the transect. As a result, we were certain all PGs collected during sampling sessions 2–3 had been deposited in the previous 8–12 days. We only completed 20 of the 34 nodes because we lacked personnel to complete the entire set within the timeframe required.

In 2011, we used the same basic field methods, however, we attempted to increase the number of transects sampled per node to 8 (N, NE, E, SE, S, SW, W, NW). Because this required more work per node, we sampled fewer nodes (14 instead of 20). Snow persistence, topography, and time constraints resulted in fewer than 8 transects on some nodes (Fig. 2). Also in 2011, surveyors were allowed to follow the first deer trail encountered irrespective of the distance from the node (e.g., within 30 m of a node) because it was determined that transect overlap was not a problem for population or density estimation. Sampling began and ended earlier in 2011 to help avoid the onset of deer movements toward summer range, and avoid the rapid advance of green up, which was believed to have reduced PG detectability during the final sampling session in 2010.

In 2010, we only collected samples found within a 1–m swath of deer trail or bearing centerlines (following methods in Brinkman et al. 2011). In 2011, to maximize sample collection, we also collected PG samples that were encountered beyond 1 m from trail centerlines because the MR and SMR methods we were using did not require that collection be limited to the swath. For determining PG density, we labeled samples according to the distance class (in 1 m increments) from the centerlines. Samples collected greater than 1 m from the centerline were not counted for



Figure 2. Location of nodes and transects for deer DNA sampling in the Pavlof study area, Chichagof Island, April–May, 2010 and 2011.

PG density analysis. Also in 2011, surveyors were allowed to collect average PGs, to increase the number of samples and thereby allow more opportunity for multiple detections of individual deer.

#### FIELD SAMPLING, GMU 3: 2012

During 2012, we conducted path sampling (Brinkman et al. 2010a) in different habitat categories to document total PG counts and fresh PG counts to measure encounter rates in GMU 3 (Fig. 1). We selected locations where habitat categories are easily accessible from the shoreline or roads. We distributed transects across the watershed and sampled at both low (0-243 m (0-799 ft)) and high (244–366 m (800–1200 ft)) elevations. We did not locate the same length of transect within each elevation zone because of differences in accessibility and snow cover. To increase our scope of inference, we sampled patches of each habitat category over a broad geographic area. We tried to keep a minimum of at least 100 m for each individual transect segment within a given habitat patch. A maximum overall transect length of approximately 4,000 m was used based on the distance one could sample in a day, but individual transect and segment lengths varied. To increase sampling efficiency, transects were created in relatively close proximity so surveyors could sample away from and then back toward the road or shoreline (Fig. 3). Transects were sampled by a single surveyor or 2-person team equipped with a compass, global positioning system (GPS), and hip chain to measure the distance traveled. Each surveyor started from predetermined start locations within a habitat patch and navigated towards predetermined end locations within the same habitat patch. Start location trees for the overall transect (but not each segment) were marked with paint and flagging. We did not flag entire transect lines in 2012 because we were not resampling those transects or conducting mark-recapture surveys as had been done on Chichagof. Encountered PGs did not need to be removed because the areas were not resampled.

As surveyors followed pre-determined bearings, they looked for deer trails and followed the trails that most closely followed the bearings. However, they needed to stay within the designated habitat patches, which were often narrow. Surveyors therefore had to use their judgment to ensure they continued to follow general bearing directions in order to stay in the habitat category they were sampling. If they were following deer trails and they deviated from the bearing by more than 90 degrees, surveyors were instructed to switch back to the bearing to avoid circling, and then continue to follow the bearing in a relatively straight line, until the next deer trail was encountered. When not following a deer trail, the route taken did not have to be perfectly straight. Rather, surveyors selected paths they believed a deer would be most likely to travel with the hope of encountering a deer trail in that area (i.e., slight deviations could be made to look for trails on ridges somewhat off of designated bearings).

Surveyors used a waypoint naming protocol, which included the node, transect, sample ID, session ID, sample quality, and distance class (in 1-m increments) to the trail or bearing centerline to log the location of PG samples. GPS identifications were recorded in GPS receivers and field notebooks. A picture was taken of the habitat where each sample was collected. To measure total PGs, we assigned a generic ID for all PGs encountered (sampled and unsampled). These waypoints were downloaded at the end of the day and identified by node and transect number.



Figure 3. Location of transects for deer DNA sampling in the GMU 3 Study Area, Kupreanof and Mitkof Islands, April–May, 2012.

To narrow habitat categories, we grouped habitats from the Terrestrial Systems GIS layer (Southeast Alaska GIS library, http://seakgis.alaska.edu; accessed 1 May 2013) into the following subcategories: 1) newcut (clearcut < 25 yrs old), 2) oldcut (clearcut > 25 yrs old), 3) scrub forest (subalpine and muskeg woodlands), 4) small productive old-growth forest (POG) (light canopy, V4H), 5) medium POG (medium canopy, V4 or V5), 6) large POG (dense canopy, V6 or V7), and 7) nonforest (water, snow, rocks, tide flats, alpine, muskeg, etc). We did not attempt to collect samples from category 7 (nonforest). Specific categories are provided in this report (Appendix A).

#### **DEER DNA ANALYSES**

Preliminary DNA analyses were performed at the University of Alaska, Fairbanks (UAF) Genetics Laboratory. All final DNA results reported here were performed at the USFS Rocky Mountain Research Station (RMRS) Genetics Laboratory (Missoula, MT). DNA was extracted from all PGs using protocols for pellets described in Brinkman et al. (2010b) using DNeasy Tissue Kits. We sent RMRS 40 tissue samples of known sex (male and female) deer from Chichagof Island, on which they tested the efficacy of various sex primers. Ideally, these primers would work for not only the Chichagof samples, but for the 2012 GMU 3 samples as well. We evaluated 14 microsatellite and two sex markers from the pool available to us (Table 1). For the Chichagof and GMU 3 samples, we ultimately used a CerZFXYf/CerZFYr sex marker plus eight microsatellite variable markers (*SBTD04, SBTD05, SBTD07, T159S, T7, T40, BM4208, and RT30*) (Table 1). We used a "multi-tube" approach for DNA analysis and genotypes were considered only when concordant allele calls were obtained.

We received individual genotypes for each sample and the number of unique individuals of deer for each population. RMRS computed a probability of identification (PID) (Waits et al. 2001) for each year of samples. PID is the probability of two different deer having identical genotypes for the markers used in the study. We report the PID for each sample population and the number of microsatellite alleles per locus per year for each study area.

#### **COMPARISON OF DNA COLLECTION METHODS**

In 2012, we investigated the efficacy of sampling DNA by conducting surface rubs of pellets using a cotton swab versus collecting whole PGs in 100% ethanol, using protocols for pellets described in Brinkman et al. (2010b). Sample IDs were identical for both swab samples and ethanol samples and pre-labeled on vials and envelopes, which were stored together prior to initiating surveys. Three separate swabs were used to sample different pellets, and then all swabs were stored in the same coin envelope. Technicians swabbed the "pinched" end of the pellet where more DNA was likely to occur. Ideally, swabs were lightly stained, but not covered in fecal matter. The swabbed samples were air-dried and stored in coin envelopes. Up to 6 individual pellets that were not swabbed were stored in ethanol and given the same sample number as those stored in coin envelopes. DNA was extracted from 40 pellets and 40 corresponding swabs stored in ethanol. We then attempted to genotype all paired samples, to compare genotyping success between the two methods.

#### **PG ENCOUNTER RATES**

We computed mean (95% confidence intervals (CI)) PG encounter rates by habitat category and PG encounter rates per length of transects within each habitat category. We calculated mean

(95% CI) PGs for a 20-m plot by habitat category to enable comparison with historical PG/20-m plot data. We also documented the average sampled PGs per 20-m for each habitat category within each watershed.

#### PATHS VERSUS STRAIGHT-LINE TRANSECT SAMPLING

In four watersheds where straight-line pellet surveys (Kirchhoff and Pitcher 1988) were traditionally conducted, we also undertook path sampling (Brinkman et al. 2010a) in the same areas to directly compare the difference in encounter rates between the two methods along the same general transect lines. Path surveys began at the end of a traditional survey, following the reverse bearing back toward the traditional transect start locations. We compared the mean number of PGs encountered per m sampled on path transects versus straight-line transects for each area in which traditional straight-line transects were conducted (3 transects/watershed). On paths, we continued to count PGs within 1 m of the transect line as Brinkman's (2010a) technique had recommended, so that we could compare our results to those recorded on POW and Chichagof. On straight-line surveys, we continued to count PGs within 0.5 m of the transect line so the results would be comparable to historic data. To reconcile the inconsistency in the width of the sampling swath, we divided the results of the path sampling by 2.

#### CHICHAGOF ABUNDANCE AND DENSITY ESTIMATION 2010–2011

We produced abundance and density estimates for 2010 and 2011. However, because sample sizes and areas sampled differed between years (2010: 20 nodes, up to 4 transects/node; 2011: 14 nodes, up to 8 transects/node), we also conducted an analysis to compare estimates across years under similar sampling schemes. For this comparison, for each year we used data from nodes 1-14 and transects 1, 3, 5, and 7 (i.e., N, E, S, W).

The two approaches we used to analyze these data are mark-recapture analysis (MR; Williams et al. 2001) and spatial mark-recapture analysis (SMR; Efford 2004, Efford et al. 2009). MR models estimate population size based on repeated detections of individual animals over a series of sampling occasions. SMR models estimate population density (i.e., number/unit area) based on repeat detections of individual animals across either time or space, or both. If there were sufficient detections, density could be estimated separately for each transect or for each node, but we had too few detections for such fine-scale estimates and estimated density for the entire study area. SMR models use information on where animals were detected whereas MR models generally do not. Both types of models account for imperfect detection (i.e., not all animals are detected during a sampling session, even though they are present on the study area) in producing estimates. For both sets of analyses (MR and SMR), we analyzed data from 2010 and 2011 separately. Also, for both analyses, we define recapture as a repeat DNA detection of an individual animal.

For our MR analyses, we used closed population models to estimate population size (Williams et al. 2001). These models assume geographic and demographic closure of the population (i.e., no animals enter or leave the study area during the study, nor are there births or deaths), which given the timing of our study, should be adequately met. The other parameter in closed-population MR models is detection probability (p; the probability that an animal is detected during a single sampling occasion), a nuisance parameter not, in this case, directly of interest, but required for accurate estimation of population size. We considered 21 models differing in what factors affected p; these factors were both categorical and continuous representations of time

(i.e., capture occasion) and sex. The sex of some identified deer could not be determined, so we used 3 categorizations of sex in different models; these were the 3-sex model (F, M, unknown) and two 2-sex models (F, M+unknown; F+unknown, M). We also used models with both time and sex. We fit models using programs MARK (Cooch and White 2014) and RMark (Laake and Rextad 2014) and selected the best model using the small-sample corrected version of Akaike's Information Criteria (AICc; Burnham and Anderson 2002).

For SMR analyses, we used the spatially explicit models of Efford et al. (2009). To use this method, we created trapsites (pellet collection sites) at 50 m intervals along each transect, and assigned each genotyped sample to the closest trapsite. We used the Split Polyline tool in ETgeowizards (http://www.ian-ko.com, accessed 1 May 2013) to divide the actual survey trackline into 50 m segments, where the final segment was 50 m + the remainder. We then created a trapsite at the centerpoint of each segment using the Feature-to-Point tool in ArcGIS, ArcToolbox (ArcGIS 10, ESRI, Redlands, CA, <u>www.esri.com</u> accessed 1 May 2013). Samples were assigned to the closest trapsite using the Near tool in ArcGIS, ArcToolbox. Multiple samples from the same deer assigned to the same trapsite and occasion were only used once in the analyses.

All statistical methods require assumptions in order to produce estimates with known characteristics and allow direct inference to larger, unsampled portions of the population of interest. Estimators that require only weak assumptions (i.e., easily met and verified) are desirable over those with strong assumptions (i.e., difficult to meet or verify); robust estimators (i.e., those insensitive to violations of assumptions) also are preferred. However, estimators that are robust and that require only weak assumptions do not exist for all situations where estimates or inference are needed. Because of this and the fact that for field-based biological data few, if any, assumptions are completely met, it is important to evaluate assumptions associated with estimators as well as we can, and study how robust the estimators are to violations of assumptions. For mark-recapture models, and other statistical methods, assumptions often can be relaxed (i.e., made weaker) using more complex models, if the data are available to support these more complex models.

For our mark-recapture models (non-spatial) (MR), important assumptions include that all animals in the population have the same detection probability for a given sampling occasion, the population is closed both demographically and geographically, and that all deer detected are correctly identified.

We relaxed the assumption of equal detectability by including models where detection probability varies by sex, which would require equal detection probability within sexes, but not between. With our node-and-spoke sampling design, animals living closer to the center of a node would potentially have higher detection probabilities than those living at the edges of a node or between nodes. However based on contemporaneous telemetry data, deer home ranges were larger than a single node and spokes and 30–40% of repeat detections of individual deer were at different nodes. And, our layout of nodes generally did not result in gaps between nodes where deer could live without detection. Other models exist that allow heterogeneity in detection probability among unknown groupings of animals, but we did not have sufficient data to implement these models. In mark-recapture models, unmodeled heterogeneity in detection probability causes negative bias in estimated population size. The reduction in population estimates with less intense sampling could, in part, be due to increased heterogeneity in capture probability with decreasing sampling effort.

The timing and duration of our sampling should reduce the likelihood of substantial violations of closure assumptions. With respect to demographic closure, our sampling was before fawns were born so there was no recruitment; there could have been some mortality during the study, but the sampling period was short making it unlikely that there was substantial mortality relative to the population size during our sampling. Likewise, geographic closure was unlikely to have been largely true. Our sampling was in the spring when much of the high elevation areas still had heavy snow cover so that migratory deer (i.e., those that use high elevation areas in the summer) likely were still on the winter range that we sampled. Repeat detections and telemetry data show some relatively long movements within the study area, so movement out of the area likely occurred for some deer, but as with mortality, probably few in relation to the population size. Open-population models that remove assumptions about closure exist, but require more data than we have. In addition, the objective of this study was to evaluate designs and analyses that are relatively cost-effective to apply widely, which would preclude collecting sufficient data for open-population models.

Because deer are identified via DNA, marked individuals cannot lose their marks. The only remaining issue here is that the DNA identifications are correct and more than one deer in the samples do not have the same DNA for the loci analyzed. Presumably, quality control at the lab precludes the first issue and the use of 8 polymorphic loci reduces the likelihood of the second to insignificance.

Spatial mark-recapture (SMR) analyses have different, but related, assumptions. These include that the detection function (i.e., g0 and sigma ( $\sigma$ )) does not differ among animals, at least within identifiable groups, and is a decreasing monotonic function of distance from the home range center, home ranges are circular with centers distributed according to a Poisson process, the analysis area is large enough such that deer with home range centers outside the analysis area have a negligible probability of being sampled, and that individuals are correctly identified.

As with the MR analyses, we included SMR models allowing detection functions to vary by sex. We have no basis to evaluate if there was heterogeneity in detection function parameters among non-identified groups, but we have no reason to expect there would be. Highly elongated home ranges result in biased estimates for SMR models. Based on detection and telemetry data, some deer movements did not meet the circular home range assumption. But most recaptures were on the same or nearby nodes, some radiotagged deer had tightly clustered, approximately circular distribution patterns, and an exponential detection function was selected from among those tried, which suggests that a circular home range assumption might be largely met for most deer in the study area. We evaluated 2 sizes of analysis area (4 or 6 km buffer around the sampled points) and found that this parameter had little influence on estimated density, suggesting we met this assumption. The concerns about correct identification are the same as with MR models.

SMR models directly estimate population density (*D*), rather than population size (*N*). Analogous to the nuisance parameter *p* in the MR models, SMR models have 2 parameters to account for detectability, g0 and  $\sigma$ . Probability of detection at an animal's home range center is g0, and  $\sigma$  models how detectability declines with distance from the home range center. In addition, for these analyses we must specify a) the parametric form of the detection function, b) the spatial extent of the estimation area, which should be large enough that animals whose home range centers are outside this area have negligible probabilities of being detected in the samples, and c) estimation grid spacing (Efford et al. 2009). We allowed g0 and  $\sigma$  to be functions of time and sex (in the same ways as in the MR models), used 3 detection functions (half-normal, exponential,

cumulative half-normal), considered 2 spatial extents (4 km and 6 km – each removed saltwater and areas above 366 m (1200 ft) elevation as non-habitat) and 3 grid spacing (200 m, 400 m, 600 m). We fit the models using the R package SECR (Efford 2012) and again selected the best model using AICc.

#### SAMPLING DESIGN AND THE EFFECTS OF SAMPLING INTENSITY AND DEER NUMBERS

#### Sampling design

Our intent with the node-and-spoke design was to estimate deer population density using distance-based trapping web analyses (Buckland et al. 2001, Link and Barker 1994). Our transect (i.e., spoke) length of 600 m was based on the average diameter of SBTD winter home range (~500 m Farmer et al. 2006, Brinkman et al. 2011). But, Buckland et al. (2001:220) stated that the density estimator is biased unless the length of the spokes in a trapping web is much longer than the home range radius of the animals under study, which was not the case for this study. Consequently, we used alternatives to the trapping web for these analyses.

#### The effect of spatial sampling intensity and number of deer

We used subsampling to investigate how spatial sampling intensity (# of transects) and deer population numbers affect the performance of our population and density estimators. These investigations can be used to help plan future deer population studies by guiding the necessary sampling intensity for various presumed densities. We conducted these subset analyses for both our MR and SMR estimation methods. For all subset analyses, we assumed the results for the analyses of the full datasets were true.

To evaluate the effect of spatial sampling intensity on our estimates, we randomly selected a subset of transects from each node for inclusion in the analyses, starting with one transect and increasing up to the maximum number of transects for each node. In 2010, we sampled 4 transects/node and in 2011 we sampled 8 transects/node; these were the maximum values as some transects were not sampled because of logistical constraints. If there were fewer transects on a node than the number being used in that particular subset analysis, all available transects were used; nodes were never dropped. This allowed us to evaluate the incremental effects of increasing sampling effort.

To evaluate the effect of lower deer numbers on the estimates, we used data from 100%, 50%, or 25% of the deer in our datasets. We felt that dropping individual deer from the analyses, rather than individual detections, would better simulate reductions in the population, rather than reductions in detectability. This approach assumes that the number of deer in our samples was proportional to the true abundance (i.e., p is not a function of density). For each of these combinations (except the 'all' analyses that used all available data to get our best estimates), we randomly selected transects and deer to be retained in the analysis and repeated this process many times (varies by combination) to get the average estimates for each configuration. The number of transects per node decreased and increased as the proportion of deer used in the analyses because computation time was higher for each SMR model. We used the best model evaluated by AIC<sub>c</sub> from the full analysis for each year as the model in the associated subset analyses.

To evaluate how decreasing transects or numbers of deer would affect the actual population or density estimates, we evaluated changes in the actual estimates, the precision of the estimates, and overall model failure using each subset. To evaluate how different subsets affected the estimate, we computed the % change between subset estimates versus the full dataset estimate. The % change was calculated as the difference between the full-data estimate and the mean subset estimate, divided by the full-data estimate.

To evaluate how each subset affected the relative precision of our estimates, we examined differences in the 95% confidence interval (CI) and the coefficient of variation (CV) between the full dataset and each subset. To calculate the % relative CI length, we substituted the length of the 95% CI for the estimate, and used the same method as we used to calculate the % change in the estimate. The CV is calculated as the mean standard error divided by the mean estimate. Failure probability is the proportion of the subset analyses that failed to provide a useful estimate (e.g., program abort, overly large estimates, or standard error of 0 or very large, indicating estimation problems). These failures were generally the result of too few repeated detections.

#### The effect of inter-node proximity

To evaluate the effect of inter-node proximity, we determined the proportion of repeat detections within nodes and estimated population density using only within-node recaptures. For this analysis, we only evaluated our basic sampling unit (i.e., a single node with 4–8 spokes), rather than multiple subsets. This simulates a sampling situation where nodes are widely scattered across the landscape such that detecting a deer at more than one node would be unlikely.

#### Estimation with a single visit

To evaluate temporal sampling intensity, we produced another SMR estimate using data only from the first sampling session. Again, we only evaluated our basic sampling unit (i.e., a single node with 4–8 spokes), rather than multiple subsets. This analysis was conducted to determine the feasibility of sampling each transect only 1 time in a season, which would greatly reduce logistical costs.

#### Assessing the assumptions of the spatial mark-recapture model

The primary assumption for the SMR model is that home ranges are unimodal and circular, at least for the period of sampling, and that home range centers are distributed according to a Poisson process. To determine the plausibility of these assumptions, we examined telemetry data from 5 radiocollared deer during our sampling window, 15 April–31 May (K. McCoy, Wildlife Biologist, ADF&G, Douglas, unpublished analysis). The deer were radiocollared in 2010–2013 with store-on-board GPS collars (Telonics Inc., Mesa, AZ). We downloaded GPS fix locations to a personal computer using Telonics software. The files were then converted to GIS databases (ArcGIS 10, ESRI, Redlands, CA) and prepared for data analysis. We plotted all GPS locations that occurred during the DNA sampling window to determine the ranges of the radiocollared deer during that time.

## Results

#### DEER DNA ANALYSES ON CHICHAGOF ISLAND

In 2010, we collected 233 PG samples in the Pavlof watershed study area. Initially in 2010, we sent the sampled PGs to the genetics lab at the University of Alaska, Fairbanks (UAF) to complete the DNA analyses, but ultimately all final analyses were conducted by Rocky Mountain Research Station in Missoula, Montana, We found that 3 of the 9 microsatellite markers used on Prince of Wales Island (POW) deer were monomorphic on Chichagof Island (Table 1). This resulted in a very low PID of 0.005. Because a PID  $\leq 0.001$  is considered the minimum acceptable level (Schwartz and Monfort 2008), we discontinued use of the monomorphic markers and included additional markers to increase the PID to an acceptable level. A new preliminary panel was originally developed at the UAF lab for the 2010 data, but use of a duplicate marker artificially inflated the PID and caused anomalies in genotyping results. Ultimately, to assure compatibility among areas and years, we analyzed all 3 years with a new panel of 8 microsatellite markers, plus a new sex marker (Table 1). The Brinkman and Hundertmark (2009) sex marker was not chosen because an artifact was being produced on the slides that made many female deer appear to be male, and artificially doubled the number of males actually in the sample. We calculated an acceptable PID from these samples (Table 2). The number of microsatellite alleles per locus per year and study area for deer in Southeast Alaska varied from 2 to 8 (Table 3).

We wanted data from all populations and years to be consistent, so we analyzed all samples and years with the same full suite of markers, even though GMU 3 had higher variation (Table 1). The final 2010 results indicated an increased number of individual deer identified and half as many males as compared to the preliminary analysis conducted at UAF. Our genotyping success in 2010 was very high (93%), with 145 deer detected overall, 52 deer detected more than once, and 18 deer detected in multiple capture sessions (Table 4). Of deer detected more than once, 20 were detected on more than one node (Table 5). Our revised sex ratio (25 M: 75 F) matches more closely to what we have seen reported in other western states, especially in hunted populations with a male-dominated harvest (biologists at Western States Deer/Elk Meeting, Missoula, MT, 2013, personal communication).

In 2011, 666 PG samples were collected in the Pavlof watershed study area. A year delay occurred in extracting the DNA from these samples, so we switched labs and then made multiple attempts to extract the degraded DNA, but genotyping success was ultimately still low (42%) (Table 6). Although DNA genotyping success was low, the number of samples collected in 2011 was 3 times as high as 2010, and was conducted over a smaller proportion of the watershed (14 of the 20 nodes done in 2010). We ended up with more individuals and recaptures over a smaller area. In 2011, we identified 189 deer (144 females, 35 males, and 10 for which we were unable to determine sex). Fifty-four deer were detected >1 time. One deer was detected 10 times, the rest were detected 2–5 times (Table 7).

Of the 189 deer detected in 2011, 35 had also been detected in 2010. Combining the deer from both years, we identified 299 individuals (Table 8). The PID for the 2011 data was a little higher than 2010, with a strong combined PID for both years combined (Table 2). Also, 4 deer that had been radiocollared on Chichagof Island appeared in our fecal DNA sampling results; 2010 only (n = 2 deer), 2011 only (n = 1 deer), both years (n = 1 deer).

Table 1. Microsatellite markers used for genetic analysis of deer in Southeast Alaska. We compared the results from Prince of Wales Island (Brinkman et al. 2010b) to the current study on Chichagof Island and GMU 3, Southeast Alaska, 2010-2012.

Marker	Prince of Wales <sup>a</sup> panel	Chichagof Island preliminary panel	Final Panel (Chichagof and GMU 3	Comments	
C89	Yes			Monomorphic <sup>b</sup>	
SBTD06	Yes			Monomorphic	
T27L	Yes			Monomorphic	
SBTD04	Yes	Yes	Yes		
SBTD05	Yes	Yes	Yes		
SBTD07	Yes	Yes	Yes		
T159	Yes	Yes	Yes	Same as Odh_O	
Τ7	Yes	Yes	Yes		
Sex marker 1	Yes	Yes		Brinkman and	
(KY1/KY2)				Hundertmark (2009)	
C273 <sup>c</sup>		Yes		Unacceptable	
Odh_O		Yes		Same as T159	
RT24				Unacceptable	
T40			Yes		
BM4208			Yes		
RT30			Yes		
Sex marker 2 (CerZFXr/CerZFYr)			Yes	Lindsay and Belant 2007	

<sup>a</sup> Brinkman et al. 2010b.

<sup>b</sup> These markers were monomorphic on Chichagof Island.
 <sup>c</sup> Removing C273 did not change the # of individuals detected and was problematic for 2011.

Locus ID	Chichagof 2010	Chichagof 2011	Chichagof, combined	GMU 3 (Kupreanof/Mitkof)
T159(O)	2.01E-01	1.96E-01	1.98E-01	2.56E-01
T7(P)	9.09E-01	8.21E-01	8.44E-01	3.87E-01
SBTD4	2.10E-01	1.91E-01	1.94E-01	1.34E-01
SBTD7	4.72E-01	5.03E-01	4.83E-01	4.14E-01
SBTD5	5.51E-01	4.89E-01	5.05E-01	3.77E-01
BM4208	2.01E-01	1.92E-01	1.95E-01	1.83E-01
RT30	4.09E-01	4.21E-01	4.11E-01	3.75E-01
T40	4.52E-01	4.06E-01	4.19E-01	2.27E-01
Sex	6.65E-01	6.94E-01	6.90E-01	4.59E-01
Cumulative	2.47 x 10 <sup>-4</sup>	1.71 x 10 <sup>-4</sup>	1.83 x 10 <sup>-4</sup>	1.48 x 10 <sup>-5</sup>

Table 2. Calculated PID for each microsatellite locus for deer in Southeast Alaska, 2010–2012.

Table 3. Number of microsatellite alleles per locus per year and study area for deer in Southeast Alaska, 2010–2012.

Locus ID	2010	2011	2010–2011	2012
T159(O)	4	5	5	3
T7(P)	2	4	4	2
SBTD4	7	7	8	6
SBTD7	2	2	2	3
SBTD5	2	2	2	2
BM4208	4	4	4	4
RT30	2	2	2	2
T40	2	2	2	3
Sex	2	2	2	2

Table 4. Individual identification of deer on NE Chichagof Island, 2010.

No. deer	No. deer detected >1 time	No. of detections per deer	No. of deer captured in >1 session	Sex ratio	Samples	Successful genotypes	Genotyping success
145	52	1–5	18	25:75 <sup>a</sup>	233	217	93%

<sup>a</sup> Sex ratio = 28 male, 97 females, 20 unknown sex.

	At 1	At 2	At 3	At 4	At 5	Total
	node	nodes	nodes	nodes	nodes	individuals
No. deer identified	125	15	4	1	0	145

Table 5. Spatial distribution of individual deer genotyped on NE Chichagof Island, 2010.

# Table 6. Genotyping success rates of deer on NE Chichagof Island by genotyping attempt and sampling session, 2011.

Session	No. samples attempt 1	Successful genotypes	Genotype success rate (%)	No. samples attempt 2	Successful genotypes	Genotype success rate (%)	Total genotypes	Overall success rate (%)
1 2 3	371 229	115 75	31 33 22	104 117 45	31 33	30 28 31	146 108 20	39 47
5 Total	666	205	23 31	43 266	14 78	31 29	29 283	44 42

Table 7. Individual identification of deer on NE Chichagof Island, 2011.

Individual deer	Deer detected >1 time	Repeat samples	No. detections per deer	No. deer detected in 2 sessions	No. deer detected in 3 sessions	No. deer detected on 2 nodes	No. deer detected on >2 nodes	Sex ratio <sup>a</sup>
189	54	95	1-10	28	5	26	3	20 males: 80 females

<sup>a</sup> Sex ratio = 35 males, 144 females, 10 unknown.

Table 8. Individual	l deer identified from	n PGs sampled on NE	Chichagof Island, 2010–2011.
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	2010	2011 new	2011 total	Detected both years	Total individuals 2010–2011
Unique deer	145	154	189	35	299
Females	97	118	144	26	215
Males	28	26	35	9	54
Undetermined	20	10	10	0	30

#### DEER DNA ANALYSES IN GMU 3: 2012

We obtained complete genotypes from 82 of the 104 samples (79%) (Table 9) collected on Kupreanof and Mitkof islands in GMU 3. Using the same 8 loci and sex marker from the 2010–2011 samples, the PID was much lower than it was on Chichagof Island (Fig. 4, Table 2), due to increased genetic variation in this population.

From these samples, 65 individuals were identified (16 males, 46 females, and 3 individuals for which we were unable to determine sex). The majority of individuals (51) were represented by 1 sample. Fourteen individuals were detected >1 time; 11 individuals were represented by 2 samples, and 3 individuals were represented by 3 samples (Table 9).



Figure 4. Cumulative PID for deer PGs sampled in GMU 3 (Kupreanof/Mitkof) based on 8 microsatellite and sex markers, 2012.

Unique individuals	Deer detected >1 time	No. detections	Samples	Samples genotyped	Genotyping success
65	14	1–3	104	82	79%

#### **COMPARISON OF DNA COLLECTION METHODS**

From the initial 40 paired samples, we obtained complete genotypes from both the swab and pellet extractions for 29 samples. Four samples failed for both methods (Table 10). For 3 PGs, we received genotyping results for the ethanol samples, but not for the swabbed samples. For 4 samples, we received genotyping results for the swapped samples, but not for the ethanol samples.

For the additional 64 samples, we were able to obtain genotypes from 32 of the swab samples, and did not attempt to obtain DNA from their paired ethanol-stored sample. However, for the 32 swab samples that failed, we decided to perform DNA extractions on the corresponding pellets stored in ethanol, to increase genotyping success. Of these, 14 samples (44%) provided genotypes.

Results category	No. of samples
Complete genotype from swab and ethanol	29
Complete genotype from ethanol/failed from swab	3
Complete genotype from swab/fail from ethanol	4
Failed genotype from swab and ethanol	4
Total	40

# Table 10. Genotyping results for deer in Southeast Alaska using different DNA extracting techniques, swab and ethanol, 2012.

#### **PG ENCOUNTER RATES**

We found that the mean encounter rate for sampled PGs ranged from 0.018 to 0.048 PG per 20-m transect segment by habitat category ( $\bar{x} = 0.026$ , CI = 0.017–0.035; Table 11). Likewise, non-sampled PGs ranged from 0.36 to 1.03 ( $\bar{x} = 0.84$  (0.70–0.98) PG per 20-m transect. However, there was substantial variability in the encounter rates among habitat patches, even of the same type, which caused wide confidence intervals on the means. Note that there were differences in the rank order of the encounter rates by habitat type for fresh samples vs. all PG.

Compared to Chichagof Island in 2010 (0.07) and 2011 (0.12), GMU 3 had fewer sampled PGs per 20-m transect in 2012 (0.03, Table 12). Likewise, Chichagof Island had more total PGs per 20-m transect in 2010 (1.93) and 2011 (1.40) compared to GMU 3 (0.86) in 2012 (Table 11). More transects were sampled on Chichagof in 2011 than in 2010, but only transects sampled in both years were included in the summary (Table 12). Although the number of PGs encountered on Chichagof on these same transects was lower in 2011, the number of samples collected was higher. This higher number of samples collected is likely the result of liberalizing our collecting protocol to include average samples. We did this because 2010 genotyping results (93%) indicated we were probably only collecting the very best samples, and might be able to get more individuals by collecting average samples.

#### PATHS VERSUS STRAIGHT-LINE TRANSECT SAMPLING

We found that PG counts were higher for path sampling compared to straight-line transects sampling when making a direct comparison between the methods. But if the results of the path sampling are halved to account for the wider swath sampled (2-m versus 1-m for the straight-line transects), results are very similar. Discussions with field crews indicate that while path sampling was designed to occur over a 2-m swath, most PGs encountered were actually within a 1-m swath. The reasons for this were twofold: 1) PGs tend to occur on or close to the trails, and taper off with distance, and 2) in densely vegetated habitats, crews could not readily detect PGs beyond that distance without slowing down to conduct detailed searches. Attempts to conduct such searches were largely unproductive and were discontinued. However, in some habitats, such as muskegs or open understories, surveyors could easily sample the 2-m swath, and so likely there was some disparity in sampling among habitat types due to their inherent characteristics and difficulties. Therefore, we present the data as both 1-m to 1-m and 1-m to 2-m comparisons (Table 13).

Habitat	No. of transect segments	Total length of segmen	Total no. of samples <sup>a</sup>	Avg no. samples per 20-m transect (95%	No. of	Avg no. PGs per 20-m transect
category	(patches)	ts (m)	(range/patch)	CI)	PGs	(95% CI)
Large	10	4,721	06 (0–3)	0.019 (0.000-0.040)	186	0.73 (0.34–1.12)
Medium	35	19,888	27 (0-4)	0.024 (0.013-0.035)	1,081	1.03 (0.81–1.26)
Newcut	11	4,895	09 (0–2)	0.048 (0.017-0.080)	133	0.72 (0.32–1.12)
Oldcut	8	3,638	06 (0-3)	0.039 (0.012–0.089)	79	0.36 (0.02–0.73)
Scrub	24	11,435	13 (0-4)	0.018 (0.002–0.034)	472	0.82 (0.53–1.12)
Small	10	4,112	04 (0-2)	0.030 (0.000-0.073)	149	0.81 (0.27–1.34)
TOTAL	98	48,689	65 (0-4)	0.026 (0.017-0.035)	2,100	0.84 (0.70-0.98)

Table 11. Comparison of the number of sampled and non-sampled PGs for each habitat category for GMU 3, 2012.

<sup>a</sup> Total samples collected for all habitat patches in this category, some of which did not genotype.

Table 12. Comparison of sampled and non-sampled PG densities per 20-m of transect on NE Chichagof Island versus GMU 3 (Kupreanof/Mitkof islands), 2010–2012.

Year	Location	Total length (m)	Samples	Non- samples	Sample PGs per 20-m transect	Total PGs per 20-m transect
2010	Chichagof <sup>a</sup>	29,363	99	2,840	0.07	1.93
2011	Chichagof <sup>a</sup>	29,363	169	2,054	0.12	1.40
2012	GMU 3 <sup>b</sup>	48,689	65	2,100	0.03	0.84

<sup>a</sup> Includes only transects sampled in both years (N, S, E, W) on nodes 1–14, 1st session only. <sup>b</sup> Does not include path-sampling results along straight-line PG survey routes.

Table 13. Comparison of encounter rates of deer PGs (non-samples only) in each watershed using straight-line transect (1-m swath) versus path sampling methods (2-m swath), GMU 3. All habitats were grouped together, 2012.

Transect type	Comparison category	Duncan (VCU 437)	Woewodski (VCU 448)	Portage (VCU 442)
Straight (1-m swath)	No. PG	170	170	146
Path (2-m swath)	No. PG	417(208) <sup>a</sup>	386 (193) <sup>a</sup>	264 (132) <sup>a</sup>
Straight	% of total PG	29%	31%	36%
Path	% of total PG	71%	69%	64%
Straight	Length of transect(m)	5,187	4,291	4,662
Path	Length of transect(m)	5,828	4,570	5,091
Straight	No. PG/20-m of transect	0.66	0.63	0.79
Path	No. PG/20-m of transect	1.43 (0.71) <sup>a</sup>	1.04 (0.52) <sup>a</sup>	1.69 (0.84) <sup>a</sup>

<sup>a</sup> Numbers were halved (in parenthesis) to account for difference in swath width. Both numbers are presented because crews indicated that while path sampling, most PG were actually encountered within a 1–m swath.

#### CHICHAGOF ABUNDANCE AND DENSITY ESTIMATION 2010–2011

#### Model selection

For the MR analysis for both years, the best model had p as a function of time; that is, each sampling period had a separate detection probability (Table 14). The model with a continuous representation of time (i.e., *Time*) was the second-best model for both years, but resulted in substantially higher (and possibly unrealistic) estimates than the best model. Sex was not an important predictor of p, but of the models with a sex effect, combining unknown-sex deer with females fit the best.

For the SMR analyses in both years, again sex was not a useful predictor, but for models with a sex effect the unknown+female grouping was best (Table 15). The exponential detection function was superior to others, indicating that detectability drops steeply with distance from the home range center. This has implications for the spacing of future sampling. Estimation grid extent and spacing, for the levels considered, had little effect on estimates, although there was some indication that the 600 m grid was too coarse. For subset analyses, we used a 4 km extent and 400 m grid, chosen on the basis of reduced analysis time. In 2010, the best model had time-specific g0 and constant  $\sigma$  (Table 15), with the reverse in 2011 (constant g0 and time specific  $\sigma$ ) (Table 16).

Table 14. Mark-recapture models used in 2010 and 2011 from data collected on NE Chichagof Island. The parameter N represents population size, the parameter of interest; SE is the standard error of the estimate. We used 3 sex categorizations: females, males, and unknowns separate (f, m, u), females separate with males and unknowns combined (f, m + u), and females and unknowns combined and males separate (f + u, m). The variables 'time' and 'Time' represent the categorical and continuous parameterizations of sampling occasions, respectively. For all variables, a '--' represents a constant effect across occasions, groups, etc. AICc is the small sample version of Akaike's Information Criteria, used to select the best model (smaller is better). Models with a sex effect for N yield multiple estimates, one for each sex, and so are not presented in the table.

Model		2010 Results			2011 Results		
N	р	AICc	$\hat{N}$	SE	AICc	$\hat{N}$	SE
		-717.3	485.0	100.7	-888.3	374.0	45.7
<sup>a</sup>	time	-808.2	379.0	73.3	-990.5	321.3	35.8
	Time	-805.8	889.0	208.9	-972.9	598.0	89.5
sex:f,m,u		-459.0			-638.9		
sex:f,m,u	time	-550.1			-741.4		
sex:f,m,u	Time	-547.1			-722.2		
sex:f,m,u	sex:f,m,u	-456.4			-636.9		
sex:f,m,u	sex:f,m,u + time	-547.6			-739.4		
sex:f,m,u	sex:f,m,u + Time	-547.6			-720.9		
sex:f,m+u		-529.6			-691.2		
sex:f,m+u	time	-620.6			-793.6		
sex:f,m+u	Time	-618.0			-776.5		
sex:f,m+u	sex:f,m+u	-528.8			-689.3		
sex:f,m+u	sex:f,m+u + time	-619.9			-791.6		
sex:f,m+u	sex:f,m+u + Time	-617.5			-773.5		
sex:f+u,m		-576.6			-723.1		
sex:f+u,m	time	-667.6			-825.4		
sex:f+u,m	Time	-665.0			-722.2		
sex:f+u,m	sex:f+u,m	-575.9			-807.4		
sex:f+u,m	sex:f+u,m + time	-667.0			-824.6		
sex:f+u,m	sex:f+u,m + Time	-664.4			-806.1		

<sup>a</sup> the best model according to AICc.

Table 15. Spatial mark-recapture models used in 2010 from data collected on NE Chichagof Island. The parameter D represents population density; SE is the standard error of the estimate. We used 3 sex categories: females, males, and unknowns (f,m,u), females separate with males and unknowns combined (f,m+u), and females and unknowns combined and males separate (f+u,m). Variables 'time' and 'Time' represent categorical and continuous parameterizations of sampling occasions, respectively. For all variables, a '--' represents a constant effect across occasions, groups, etc. AICc is the small sample version of Akaike's Information Criteria, used to select the best model (smaller is better). Models with sex effect for D yield multiple estimates, one for each sex, and so are not presented in the table.

		Model				2	2010 resu	lts
D	g0	σ	Dist. <sup>a</sup>	Grid	Extent	AICc	$\hat{D}$	SE
	Time		exp.	200 m	4 km	2,130.6	0.044	0.00003
	time		exp.	400 m	4 km	2,130.0	0.044	0.0061
	time		exp.	600 m	4 km	2,131.9	0.044	0.0061
	time		exp.	200 m	6 km	2,130.7	0.043	0.0061
	time		exp.	400 m	6 km	2,130.1	0.044	0.0061
	time		exp.	600 m	6 km	2,130.5	0.044	0.0061
<sup>b</sup>	time		exp.	400 m	4 km	2,130.0	0.044	0.0061
	time		half	400 m	4 km	2,151.8	0.036	0.0048
	time		c.half	400 m	4 km	2,139.1	0.037	0.0054
			exp.	400 m	4 km	2,242.8	0.044	0.0061
	Time		exp.	400 m	4 km	2,139.0	0.044	0.0061
		Time	exp.	400 m	4 km	2,141.2	0.041	0.0057
	Time	Time	exp.	400 m	4 km	2,138.5	0.044	0.0063
		time	exp.	400 m	4 km	2,132.4	0.043	0.0060
	time	time	exp.	400 m	4 km	2,133.8	0.045	0.0065
sex:f,m,u	sex:f,m,u		exp.	400 m	4 km	2,499.7		
sex:f,m,u		sex:f,m,u	exp.	400 m	4 km	2,498.5		
sex:f,m,u	sex:f,m,u	sex:f,m,u	exp.	400 m	4 km	2,492.4		
sex:f,m+u	sex:f,m+u		exp.	400 m	4 km	2,428.4		
sex:f,m+u		sex:f,m+u	exp.	400 m	4 km	2,429.1		
sex:f,m+u	sex:f,m+u	sex:f,m+u	exp.	400 m	4 km	2,431.1		
sex:f+u,m	sex:f+u,m		exp.	400 m	4 km	2,381.8		
sex:f+u,m		sex:f+u,m	exp.	400 m	4 km	2,338.9		
sex:f+u,m	sex:f+u,m	sex:f+u,m	exp.	400 m	4 km	2,378.0		
sex:f+u,m	time	sex:f+u,m	exp.	400 m	4 km	2,271.2		
sex:f+u,m		sex:f+u,m+time	exp.	400 m	4 km	2,273.4		
sex:f+u,m	time	sex:f+u,m+time	exp.	400 m	4 km	2,274.9		

<sup>a</sup> Detection functions: exp. = exponential, half = half normal, and c. half = compound halfnormal.

<sup>b</sup> The best model according to AICc.

Table 16. Spatial mark-recapture models used in 2011 from data collected on NE Chichagof Island. The parameter D represents population density; SE is the standard error of the estimate. We used 3 sex categorizations: females, males, and unknowns separate (f,m,u), females separate with males and unknowns combined (f,m+u), and females and unknowns combined and males separate (f+u,m). The variables 'time' and 'Time' represent the categorical and continuous parameterizations of sampling occasions, respectively. For all variables, a '--' represents a constant effect across occasions, groups, etc. AICc is the small sample version of Akaike's Information Criteria, used to select the best model (smaller is better). Models with a sex effect for D yield multiple estimates, one for each sex, and so are not presented in the table.

	Model					20	)11 Resu	lts
D	g0	σ	Dist. <sup>a</sup>	Grid	Extent	AICc	$\hat{D}$	SE
b		time	exp.	200 m	4 km	2,803.2	0.122	0.0143
		time	exp.	400 m	4 km	2,804.2	0.119	0.0140
		time	exp.	600 m	4 km	2,806.0	0.117	0.0136
		time	exp.	200 m	6 km	2,803.2	0.122	0.0143
		time	exp.	400 m	6 km	2,804.2	0.119	0.0140
		time	exp.	600 m	6 km	2,810.3	0.119	0.0137
		time	exp.	400 m	4 km	2,804.2	0.119	0.0140
		time	half	400 m	4 km	2,846.0	0.104	0.0120
		time	c. half	400 m	4 km	2,815.0	0.110	0.0128
			exp.	400 m	4 km	2,887.7	0.118	0.0139
	Time		exp.	400 m	4 km	2,822.9	0.118	0.0139
		Time	exp.	400 m	4 km	2,815.4	0.121	0.0141
	Time	Time	exp.	400 m	4 km	2,817.3	0.121	0.0141
	time		exp.	400 m	4 km	2,811.1	0.119	0.0140
		time	exp.	400 m	4 km	2,804.1	0.119	0.0140
	time	time	exp.	400 m	4 km	2,807.7	0.120	0.0142
sex:f,m,u	sex:f,m,u		exp.	400 m	4 km	3,144.3		
sex:f,m,u		sex:f,m,u	exp.	400 m	4 km	3,134.6		
sex:f,m,u	sex:f,m,u	sex:f,m,u	exp.	400 m	4 km	3,126.7		
sex:f,m+u	sex:f,m+u		exp.	400 m	4 km	3,091.9		
sex:f,m+u		sex:f,m+u	exp.	400 m	4 km	3,080.6		
sex:f,m+u	sex:f,m+u	sex:f,m+u	exp.	400 m	4 km	3,078.2		
sex:f+u,m	sex:f+u,m		exp.	400 m	4 km	3,064.9		
sex:f+u,m		sex:f+u,m	exp.	400 m	4 km	3,056.0		
sex:f+u,m	sex:f+u,m	sex:f+u,m	exp.	400 m	4 km	3,057.4		
sex:f+u,m	time	sex:f+u,m	exp.	400 m	4 km	2,979.8		
sex:f+u,m		sex:f+u,m+time	exp.	400 m	4 km	2,974.6		
sex:f+u,m	time	sex:f+u,m+time	exp.	400 m	4 km	2,978.6		

<sup>a</sup> Detection functions: exp.= exponential, half = half normal, and c. half = compound half-normal. <sup>b</sup> The best model according to AICc.

#### Population and density estimates

The estimated population size, based on MR analysis, was larger in 2010 than 2011 (Table 17), but the area sampled in 2010 also was substantially larger. Using data from a comparable number of nodes and transects, the annual estimates were similar. Based on the SMR analyses, population density was much higher in 2011 than in 2010 (Table 17). Because density incorporates the area sampled, density should be less affected by differences in the area sampled than MR estimates. But as with the MR estimates, the density estimates based on comparable sampling between years (2010a and 2010a) yields more similar estimates (Table 17). For density estimates based on a single survey of each transect (2010b and 2011b), the 2010 estimate was essentially the same as for the full data set, while the 2011 estimate was less than the full data estimate, though still larger than the 2010 estimate (Table 17). As expected, standard errors of the estimated densities were larger for the single visit analysis than the full data analyses. More samples were collected during the third sampling session in 2011 than in 2010, which may account for some of the difference. Density estimates for each year were 3-6 times higher when recaptures were restricted to a single node, which highlights that the scale of a single node (i.e., a 600 m radius circle) inadequately represented the movement, and hence detection function, of deer in this study area, resulting in overestimation of density.

MR models	Ν	SE	CV	95% CI	Nodes	Transects/node
2010	363	76.5	0.21	255-568	20	4
2011	321	35.8	0.11	266-410	14	8
2010 <sup>a</sup>	235	49.5	0.21	167-371	14	4
2011 <sup>a</sup>	219	43.0	0.20	159–336	14	4
b)						
SMR models	D (deer/ha)	SE	CV	95% CI	Nodes	Transect/node
2010	0.044	0.0061	0.14	0.034-0.058	20	4
2011	0.119	0.0140	0.12	0.095-0.150	14	8
2010 <sup>a</sup>	0.065	0.0126	0.19	0.047-0.090	14	4
2011 <sup>a</sup>	0.084	0.0152	0.18	0.059-0.120	14	4
2010 <sup>b</sup>	0.047	0.0097	0.21	0.032-0.070	20	4
2011 <sup>b</sup>	0.071	0.0142	0.20	0.048-0.105	14	8
2010 <sup>c</sup>	0.246	0.041	0.17	0.178-0.241	20	4
2011 <sup>c</sup>	0.363	0.510	0.14	0.276-0.478	14	8

Table 17. Population size (N) (a) and density estimates (D) (b) for the NE Chichagof study area, 2010 and 2011.

a)

 $^{\rm a}$  2010 and 2011: 'comparable' analysis using only nodes 1–14 and transects 1, 3, 5, 7 for the MR and SMR analysis.

<sup>b</sup> 2010 and 2011: analysis using all nodes and transects, but first sampling occasion only.

<sup>c</sup> 2010 and 2011: analyses using all nodes and transects, but only repeated detections withinnodes (i.e., no multi-node recaptures).

#### SAMPLING DESIGN AND THE EFFECTS OF SAMPLING INTENSITY AND DEER NUMBERS

#### Sampling design

The node and spoke design we implemented used 20 nodes with a maximum of 4 transects/node in 2010, and 14 nodes with a maximum of 8 transects/node in 2011. Some nodes had less than the maximum due to topographic limitations (Fig. 2, Fig. 3).

#### Spatial sampling intensity and deer numbers

To evaluate the effect of spatial sampling intensity, we reduced the number of transects sampled/node. To evaluate how different numbers of deer on the landscape would affect our estimates, we looked at changes in our estimates based on 100%, 50% and 25% of deer identified. For each year, we used different combinations of numbers of transects and population reductions in our analyses (Table 18).

# Table 18: Quantity of transects used and the proportion of deer included in MR and SMR subsets on NE Chichagof Island, 2010–2011.

Transects/node	2010	2011
8		All <sup>a</sup> , 50, 25
6		100, 50, 25
4	All <sup>a</sup> , 50, 25	100, 50, 25
2	100, 50, 25	100, 50, 25
1	100, 50, 25	100, 50, 25

<sup>a</sup> 'All' indicates this subset actually includes the maximum amount of data (i.e., all transects and deer).

MR estimates were sensitive to reduced sampling effort (i.e., fewer transects/node), showing, on average, negative % change in the estimate as the number of sampled transects decreased (Fig. 5); this was evident to some extent irrespective of the deer density (as represented by reductions in the proportion of deer used in the analyses). SMR density estimates were much less affected by reduced sampling, except when only 1 transect/node was used (Fig. 5). The 2010 density estimates with reduced sampling displayed a positive bias due to a skewed distribution of the replicate density estimates, so the % change in the median estimate is presented instead.

Relative confidence interval lengths increased with less intensive sampling for both MR and SMR estimates, indicating less precision with fewer transects (Fig. 6). However, the reduction in the confidence interval lengths slows with more than 4 transects sampled/node. Similarly, CVs for both MR and SMR estimates increased with reduced sampling (Fig. 7), with improvement slowing beyond 4 transects/node, at least at higher deer abundance. For a given level of sampling intensity, CVs were higher for lower abundance deer populations (Fig. 7).

With reduced sampling and lower deer abundance, both MR and SMR models could fail (i.e., not produce an estimate) because there would be too few repeat detections of individual deer. Four transects/node reduced model failure to 0 in almost all cases (Fig. 8). For low abundance

populations, failure probability often was high when using only 2 transects/node for both analysis methods. In contrast, failure probabilities were <10% for higher abundance populations, although only sampling 1 transect/node yielded high failure probabilities in almost all cases (Fig. 8).



a)





Figure 5. The % change in the (a) population abundance estimate (N) and % change in the (b) population density estimate (D) for 2010 and 2011 using 100%, 50%, and 25% of deer identified.



Figure 6. The % relative confidence interval length (CI) of the 2010 and 2011 estimate for the non-spatial (MR) and spatial (SMR) analyses.



b)



Figure 7. Abundance precision (CV) for (a) the non-spatial (MR) and (b) spatial (SMR) analyses given different numbers of transects and percent deer used, 2010 and 2011.



b)



Figure 8. Model failure for (a) the non-spatial (MR) and (b) spatial (SMR) analyses given different numbers of transects and percent deer used, 2010 and 2011.

For each analysis, including the subset analyses, we calculated the number of recaptures used in computing estimated density (Table 19). For each deer, the number of recaptures is the number of detections minus one; repeat samples of the same deer at the same trapsite (i.e., 50 m segment of a transect) are not counted as recaptures, even if the samples were from different sampling sessions. Estimation of the detection function using spatial methods is based on the sample of distances between detections of the same animal (Efford 2004). As such, repeat samples from the

same trapsite do not yield distances useful in the estimation. A rule-of-thumb of a minimum of 20 recaptures is advocated for using spatial mark-recapture models (Efford et al. 2009). Some of our subset analyses often had fewer recaptures than suggested, especially with less intensive sampling and lower deer numbers (Table 19).

#### The effect of inter-node proximity

We also calculated the number of recaptures within nodes (i.e., detections of the same deer at different nodes were not counted as recaptures). This is useful for planning studies where clusters of transects are far from one another such that the recaptures at >1 cluster would be unlikely. Using only within-node recaptures would reduce the number of recaptures by 30-40%, which should be considered when planning future projects. It also is important that the total number of recaptures across deer does not translate directly to the number of movement distances used in estimating detectability. For example, 5 deer detected twice each (i.e., 1 recapture/deer) would yield 5 distances, but 2 deer detected 5 times each (i.e., 4 recaptures/deer) would yield 20 distances for the analysis.

The number of distances/deer is  $\binom{n}{2} = \frac{n*(n-1)}{2}$ , where n is the number of detections. So the amount of data available for estimating the detection function varies with how many times each deer is detected, not just the total number of recaptures. But, the number of recaptures is a useful relative measure of the data available. Density estimates based only on within-node recaptures resulted in much higher estimates than when all detections were used (Table 17).

#### Estimation with a single visit

We found that a single capture session yielded SMR estimates that were relatively similar to estimates using the entire dataset, but the similarity varied by year (Table 17). The 2010 estimates (0.044 full data versus 0.047 for the  $1^{st}$  session only) were actually more similar than the 2011 estimates (0.119 full data versus 0.071 for the  $1^{st}$  session only).

Although not part of our original objectives, we also explored the possibility of using the 2012 data collected in GMU 3 (where deer density is lower) to produce a single-session capture-recapture analysis, recognizing that the transect layout was not designed for producing a precise density estimate. Unfortunately, only 14 of the pairs of detections were far enough apart to be considered a 'capture and recapture'. Deer were generally detected multiple times on the same transect segment, not adjacent transect segments.

#### Assessing the assumptions of the spatial mark-recapture model

We found that locations from 3 of the 5 GPS radiocollared deer were unimodal and roughly circular, but locations from the remaining 2 deer were bimodal and/or elongated (Fig. 9). Of the deer that were bimodal, roughly a month was unimodal, and then the deer started to move, presumably toward summer range. The date on which each deer moved varied within the 15 April–31 May window. These data suggest that assumptions may be met for all deer if sampling is conducted over a shorter time frame.

# Table 19. Number of recaptures of deer within various modeling scenarios for NE Chichagof Island, 2010 and 2011. We changed the number of deer present and the number of transects within nodes and then looked at the number of recaptures with the given parameters.

	Mo	del paramete	rs	А	All recaptures Within-node recaptures		Proportion within nodes			
Year	Nodes	Transects	% deer	Median	Min	Max	Median	Min	Max	Median
2010	20	4	100	61			37			0.61
	20	4	50	30	17	41	19	11	26	0.63
	20	4	25	15	6	24	9	3	16	0.60
	20	2	100	21	13	31	13	7	20	0.62
	20	2	50	10	4	19	6	1	12	0.60
	20	2	25	5	0	14	3	0	10	0.60
	20	1	100	7	1	15	5	0	10	0.71
	20	1	50	3	0	10	2	0	8	0.67
	20	1	25	2	0	7	1	0	5	0.50
2011	14	8	100	85			54			0.64
	14	8	50	42	30	54	26	16	38	0.61
	14	8	25	22	10	38	14	4	28	0.64
	14	6	100	62	47	73	39	32	47	0.63
	14	6	50	32	14	48	21	5	34	0.65
	14	6	25	15	6	32	10	2	26	0.67
	14	4	100	36	21	51	22	12	32	0.61
	14	4	50	18	7	31	11	4	21	0.61
	14	4	25	9	1	21	5	0	15	0.56
	14	2	100	10	4	22	7	2	16	0.70
	14	2	50	6	0	17	4	0	12	0.67
	14	2	25	2	0	11	2	0	7	1.00
	14	1	100	3	0	14	2	0	10	0.67
	14	1	50	1	0	7	1	0	5	1.00
	14	1	25	1	0	4	1	0	3	1.00



Figure 9. Locations of 5 deer near nodes 1–15, during the DNA sampling window of 15 April–31 May, 2010 and 2011.

# Discussion

#### **DEER DNA ANALYSES**

Although genotyping success was low in 2011 (42%) due to a year delay in DNA extraction and a further delay in sample genotyping, it was high in 2010 (93%) and 2012 (79%), indicating that our field protocols for sampling and preserving samples were working well. Processing of samples in 2012 was delayed 3 months due to the logistics associated with switching to a new lab, which may account for the slightly lower genotyping success rates in 2012 as compared to 2010. We recommend that the DNA from field samples be extracted as quickly as possible after sampling, to fully preserve the DNA, especially if samples cannot be genotyped for several months.

#### COMPARISON OF DNA COLLECTION METHODS

The advantages of dried-surface swabs over storage is ethanol is that dried swabs are much easier to handle and store, they have less restrictive national and international shipping, and they may have a longer shelf life before they need to have the DNA extracted (David Paetkau, Wildlife Genetics International, personal communication). One potential disadvantage is that more skill is required on the part of the sampler not to under-swab or over-swab the fecal material, both of which could result in a lack of adequate DNA (Wildlife Genetics International, http://www.wildlifegenetics.ca/; accessed 1 May 2012).

The overall genotyping success between the two methods was similar. However, our data also indicated that if one method fails for a group of samples, approximately 40% of those that failed may produce a genotype using the other method. Therefore, use of both methods should be considered. However, we also caution that DNA should be extracted from ethanol samples immediately, even if all samples will not ultimately be genotyped, because the DNA will start to degrade. It should be noted that although the cost of DNA extraction is relatively low (~\$5/sample), genotyping paired ethanol samples will increase the cost of DNA analyses substantially (~\$80/sample). The extent of the total cost increase will therefore depend on the success of the primary method used, because additional analysis is only needed on those samples that failed the first genotyping attempt.

#### PELLET GROUP ENCOUNTER RATES

The encounter rate of sampled PGs was much lower in 2010 than 2011 on Chichagof Island because we collected more average PGs in 2011. Unfortunately, we were unable to evaluate how genotyping success may have changed due to collecting more average PGs because the samples were not genotyped for over a year. The delay led to degradation of the DNA and low genotyping success. However, it may be worth lowering PG collection standards in GMU 3 (Kupreanof/Mitkof islands) in order to evaluate if we can increase sample size and multiple detections without encountering poor genotyping success or unreasonably high associated costs.

The number of non-sampled PGs encountered on Chichagof Island in 2010 was somewhat higher than what was encountered in 2011. This could indicate less activity on these transects by deer in 2011, but could also be the result of lower PG persistence rates in 2010. Degradation rates of PGs is believed to vary depending on habitat, diet, and the average climatic condition in different locales, but can also be affected by year-specific weather variation (i.e., snow, rain, and freezing temperatures) at specific locations (Fairbanks 1979, Fisch 1979, Harestad and Bunnel 1987). In

Southeast Alaska, PG persistence has been shown to range from 4 months to 11 months depending on local environmental conditions (Rose 1982, Kirchhoff 1990, Farmer and Person 1999). When conducting spring PG counts, we estimate PG persistence in old growth to be approximately 7 months (over-winter depositions), but the reality is that this can vary from year to year and location to location. To accurately account for this, PG persistence would need to be measured at specific sites of interest. In our study area, the 2010 PGs were removed along transects, so the only PGs available to be counted in 2011 were those deposited after the final sampling session in 2010. If PG persistence was longer than a year at this location, the 2010 counts might have been higher because older PGs had not been removed.

We found substantial variation in encounter rates, both between and within habitat types. However, the among-habitat patterns differed for fresh samples and all PGs. Differences in the rank order of encounter rates by habitat type for fresh samples versus all pellet groups could reflect differing habitat use between winter and spring. For example, medium habitat had the highest mean encounter rate for all PGs but a relatively low rate for samples. In contrast, newcut and oldcut types had high sample encounter rates but low total PG encounter rates. This suggests that deer use medium habitat patches more heavily in the winter and use newcut and oldcut patches more in the spring, when the samples were collected. Because PGs occur in all habitats, sampling all categories helps assure a representative sample of deer use and densities (Brinkman et al. 2011).

#### PATH VERSUS STRAIGHT-LINE TRANSECT SAMPLING

We had hoped to be able to quantify the extent PGs may be more abundant along path-sampled deer trails as opposed to along straight-line PG transects to evaluate potential differences in sampling efficiency between the 2 methods. However, because of the pattern of PG occurrence on and along deer trails and inconsistencies with detectability for a 2-m swath among different habitat types, our results for this comparison are not valid. Our crews indicated that they encountered a pattern in PGs along deer trails. PGs are more dense on or adjacent to the trail, and taper off with distance. In the future, for a more robust comparison of the number of PGs encountered on path versus straight-line transects, we recommend using a 1-m swath for greater efficiency, and using the exact same-sized swath for each method. Crew observations suggest that using a 1-m swath along deer trails should yield densities similar or only slightly higher than if a 2-m swath were used, and the smaller swath assures greater ease of consistency in all habitat types and eliminates the need for extensive searches in heavily vegetated habitats.

#### CHICHAGOF ABUNDANCE AND DENSITY ESTIMATION 2010–2011

We evaluated various sampling and analysis options for DNA-based population studies of deer in southeastern Alaska. Because we used a variety of approaches, we also produced multiple estimates of population size and density; differences in estimates from the various study methods are useful for evaluating and refining these relatively new methods of population study. However, comparing deer population size and density between years requires some care.

Using all the data with the MR analysis, the population estimate for 2010 was higher than for 2011 (Table 17). However, a larger area (and more nodes) was sampled in 2010 than in 2011. If only the same 14 nodes and transects sampled in both years are used in the analysis, the population estimates are much closer. In 2010, the 35% reduction in the estimated population size between the full analysis and the 'comparable' analysis corresponds relatively well with the

30% reduction in the number of nodes (i.e., area) sampled in 2011. A similar argument can be made for the 32% reduction in the 2011 estimates because, whereas the number of nodes (i.e., area) sampled did not differ between the full and comparable analyses, the comparable analysis used fewer transects for 2011. The results of the comparable analysis agree with the results of the subset analysis of the 2011 data, which showed that MR-based estimates were sensitive to reduced sampling effort with, on average, a 21% drop in estimated population size with 4 transects per node, compared to the full analysis with 8 transects/node (Fig 5a). Although we did not sample 8 transects/node in 2010, our subset results seem to indicate that the 2010 estimate would have been higher had we done so.

In contrast, SMR-based density estimates are less sensitive to changes in sampling intensity, showing an average drop of 11% in estimated population density when data from 4 transects are used compared to the full analysis with 8 transects/node in 2011 (Fig. 5b); analysis of the 2010 data suggest even less sensitivity to changes in sampling intensity. Although the 2010 estimated density (0.044 deer/ha; 4.4 deer/km<sup>2</sup>;11.2 deer/mi<sup>2</sup>) was less than half of the 2011 estimated density (0.119 deer/ha; 11.9 deer/km<sup>2</sup>; 30.5 deer/mi<sup>2</sup>) with full dataset analyses, the density of deer likely did not necessarily double between years (Table 17). Rather, it seems more likely that density is not uniform across the nodes. For example, if the density on nodes 15–20 were lower than the density of the area sampled by nodes 1–14, the overall 2010 density estimate would be less, since it included the additional nodes. Using the comparable analyses with the same nodes and transects both years yields more similar results between years (Table 17, 2010a and 2011a), indicating nodes 15–20 might have had a lower density. As one can see, interpretation of these data can be complex.

The comparable analysis gives the best indication of the actual percent change in density (29%) between years, 0.065 deer/ha (6.5 deer/km<sup>2</sup>; 16.6 deer/mi<sup>2</sup>) in 2010 to 0.084 deer/ha (8.4 deer/km<sup>2</sup>; 21.5 deer/mi<sup>2</sup>) in 2011, for the area sampled by nodes 1–14. However, the SMR estimate using the full data set gives the best absolute density estimates (0.044 deer/ha; 4.4 deer/km<sup>2</sup>; 11.3 deer/mi<sup>2</sup> in 2010 and 0.119 deer/ha; 11.9 deer/km<sup>2</sup>; 30.5 deer/mi<sup>2</sup> in 2011) for the overall area sampled each year. Nevertheless, it is possible that the 2010 full data estimate, which is based on 4 transects/node, could have a slight negative bias (Fig. 5), which would underestimate the density.

Our estimates are comparable to estimates of deer densities of 9.4 deer/km<sup>2</sup> (SE = 1.46) on managed lands (24.0 deer/mi<sup>2</sup>) and 12.2 deer/km<sup>2</sup> (SE = 1.37) on unmanaged lands (31.2 deer/mi<sup>2</sup>) of Prince of Wales Island (Brinkman et al. 2011). We are not aware of any other estimates of deer densities in Southeast Alaska.

#### SAMPLING DESIGN AND THE EFFECTS OF SAMPLING INTENSITY AND DEER NUMBERS

#### Sampling design

Our node-and-spoke sampling design worked well and produced good estimates (CV < 20%). However, the original reason we used the node-and-spoke design was to employ a distance-based trapping web analysis, which we did not ultimately use because our spoke transect lengths would have needed to be much longer than the home range radius of a deer (Buckland et al. 2001). As a result, the node-and-spoke design was unnecessary, and logistically inefficient because surveyors lost considerable field time having to backtrack to node center points to establish transects, as well as while moving between transects and nodes on subsequent visits. Other designs should be considered. Surveying out and back to your point of origin (on a road or shore) in the form of a square, rectangle, or triangle would likely be a more logistically efficient sampling design. Transects of these designs could be clustered within an overall area of interest. Alternatively, path surveying could be conducted within a square or rectangular area such that surveyors traverse back and forth across the area (i.e., survey up to a point, across to another point, down to a point, across, up, across, etc), which might allow high efficiency as well as density of sampling. Under this scenario, the shorter the across transects, the higher the survey density.

#### The effect of spatial sampling intensity and numbers of deer

Efford et al. 2009 provides a rule-of-thumb that >20 repeat detections are necessary for good performance of SMR methods. This rule, along with our results and encounter rate estimates, can be used to plan sampling intensities for future projects. Sampling intensity includes the number of clusters, the number of transects/cluster, and the number of repeat visits to transects or clusters.

#### The effect of inter-node proximity

Using only within-node recaptures resulted in extreme overestimates of population density. This indicates that if the transect clusters are widely spaced (unlike our study) the clusters should cover areas substantially larger than a 600 m radius circle. Another potential design, where individual plots are sampled within a study area, has been described (Efford 2011, Goode 2011). This design is reasonable, but it seems this method would result in lost opportunity to collect fresh pellets while moving between plots.

#### Estimation with a single visit

Even a single visit to each transect seemed to yield relatively good results for the 2011 data on Chichagof, but the density of transects and nodes within that watershed were substantially higher than in GMU 3. At the lower densities that appear to exist in GMU 3, multiple capture sessions and a higher sampling intensity (i.e., more transects) are likely necessary for successfully conducting mark-recapture.

#### Assessing the assumptions of the spatial mark-recapture model

Other assumptions of SMR need to be carefully considered and have to be approximately true to get minimally biased estimates. The primary assumption is that home ranges are unimodal and circular, at least for the period of sampling, and that home range centers are distributed according to a Poisson process. We examined the assumption of home range circularity by looking at data from radiocollared deer and found that the assumption may be true if sampling is conducted over a short time frame. For future studies, perhaps two 10-day sampling sessions or three 7-day sessions would result in more circular movement patterns as assumed by the model. Because SMR methods are relatively new, the robustness of the procedures have not been fully explored, but Ivan et al. (2013) reported little bias in SMR-based density estimates except when home ranges were highly elongated, especially when home ranges were large relative to the study area size.

# Recommendations

In planning for future fecal DNA-based population estimation projects, several recommendations follow from our results.

We recommend using the microsatellite panel we have developed as a starting place for DNA mark-recapture analyses in Alaska. However, because we found that genetic variation can vary substantially by location, further work is needed to determine whether this panel can be used across Southeast Alaska or Alaska as a whole.

We recommend that researchers who are not limited by transportation and shipping concerns of ethanol collect DNA using both surface swab and pellets-in-ethanol storage methods because a combined approach may consistently yield higher genotyping rates than either method alone.

We recommend that all habitat categories be sampled proportionately on the landscape, as deer used all habitat categories and fresh PGs were encountered in each. We also recommend that future evaluation of PG densities along path or straight-line transects use a 1-m swath, as it should provide more consistent sampling in all habitat types.

We do not recommend the use of our node-and-spoke sampling design, as it was logistically inefficient. Instead, we recommend using a method where surveyors do not have to do a lot of backtracking, such as square, rectangular, triangular, or grid (up, across, down, across, up, across, etc.) patterns.

For our situation, SMR methods have as good or better properties than MR methods, particularly with respect to sampling intensity, as illustrated by the percent change in the estimates, leading us to recommend SMR methods over MR methods.

We recommend clustering transects, rather than uniformly distributing them across the landscape, to obtain sufficient repeat detections for population size or density estimation. A sampling design of four 600 m transects/cluster, or roughly 2,400 m of transects for a 600 m radius circle, gave generally satisfactory results (i.e., little % change in the estimate, good precision, low model failure) when there was little space between clusters. This may not be true for areas with very low deer abundance, where more transects might be needed. Theoretically, randomly distributing transect clusters over a large area should yield reasonable results for estimating average population density across the area. Although SMR assumes that detectability relative to home range centers does not differ among animals, not all animals must be detectable (e.g., inhabit a sample cluster), which would allow gaps between the clusters of transects leading to more efficient sampling of larger study areas. But given the relatively large proportion of between-node detections (Table 19) and much higher density estimates using only within-node recaptures, we do not recommend widely spaced single clusters if they cover the same area as our node and spoke clusters. Clusters of transects would either need to cover a substantially larger area than a 600-m radius circle or clusters would need to be close enough to detect individual deer moving between clusters (e.g., groups of transect clusters).

Although the SMR analyses indicate that deer are relatively sedentary during late spring, due to a few long movements by deer within our study areas as well as contemporaneous telemetry data, we recommend that sampling periods should be as short as practical (e.g., 7–10 days) to best meet the assumption of home range circularity and minimize the likelihood of longer movements. We do not recommend using a single sampling session where deer population density is low. However, where density is higher (e.g., at the level of the Chichagof study area),

single sampling sessions can yield good estimates, assuming that a sufficient number of transect clusters are sampled (e.g., 14 closely-spaced nodes (clusters) with up to 8 transects each).

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Habitat category	GIS_CODE	Description
No data	1	nonforest
Large POG (productive old growth), V67	6	large
Medium POG, V5-N	5	medium
Medium POG, V5-S	5	medium
Medium POG, V4-N	5	medium
Medium POG, V4-S	5	medium
Medium POG, V5-H	5	medium
Small POG, V4-H	4	small
Large POG, V67 (karst)	6	large
Medium POG, V5 (karst)	5	medium
Medium POG, V4 (karst)	5	medium
Large POG, V67 (valley floor)	6	large
Medium POG, V5 (valley floor)	5	medium
Medium POG, V4 (valley floor)	5	medium
Logged (<25 yr)	2	newcut
Logged (>25 yr)	3	oldcut
Logged (unknown)	3	oldcut
Sub-alpine forest	1	scrub
Sub-alpine forest (wet)	1	scrub
Conifer (YG)	1	scrub
Conifer (unknown)	1	scrub
Scrub forest (wet)	1	scrub
Muskeg woodland	1	scrub
Deciduous forest	1	nonforest
Shrubland	1	nonforest
Slide zone	1	nonforest
Alpine tundra	1	nonforest
Muskeg meadow	1	nonforest
Herbaceous (other)	1	nonforest
Nonforest vegetation	1	nonforest
Nonforest vegetation (wetland)	1	nonforest
Salt marsh	1	nonforest
Aquatic bed	1	nonforest
Unconsolidated / tide flat	1	nonforest
Estuary (other)	1	nonforest
Snow	1	nonforest
Rock	1	nonforest
Unvegetated (other) and water	1	nonforest

**Appendix A.** Habitat categories used, based on a reclassification of terrestrial systems landcover (Southeast Alaska GIS library, http://seakgis.alaska.edu; accessed 1 May 2013).

#### 44 Assessing population estimation protocols for Sitka black-tailed deer using DNA from fecal pellets.

