Wildlife Research Final Report

Assessing Population Trends of Deer in Southeast Alaska using a DNA-based Approach

A General Guide, Version 1.0

Todd J. Brinkman, David K. Person, Kris J. Hundertmark

December 2010

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ADF&G Division of Wildlife Conservation, P.O. Box 110024, Juneau, Alaska 99811-5412; email dfg.dwc.publications@alaska.gov; phone (907) 465-4272.

Cover Photo: Radiocollared adult female Sitka black-tailed deer and her radiocollared fawn on Prince of Wales Island. Photo by Steve Bethune.

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Todd J. Brinkman, Ph.D.
University of Alaska Fairbanks
211 Irving, Building 1
Fairbanks, Alaska, 99775

David K. Person, Ph.D.
Alaska Department of Fish and Game
Division of Wildlife Conservation
2030 Sea Level Dr. #205
Ketchikan, AK 99901

Kris J. Hundertmark, Ph.D.
University of Alaska Fairbanks
211 Irving, Building 1
Fairbanks, Alaska, 99775

December 2010

Alaska Department of Fish and Game
Division of Wildlife Conservation
P. O. 110024
Juneau, AK 99811

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OBJECTIVE

The objective of this guide is to provide information that will help wildlife biologists in Southeast Alaska (and other places where densely forested environments hinder data collection through direct observation) design and implement genetic-based studies of Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) that use DNA extracted from fecal pellets collected in the field. We hope this guide will stimulate interest among biologists in the use of non-invasive DNA techniques to address population-level questions about deer in Southeast Alaska. We assume the reader has a general understanding of the ecological relations between deer and coastal temperate rainforests in Southeast Alaska.

BACKGROUND

For over a half-century, management programs in North American and elsewhere relied on fecal pellet-group counts (PG counts) to estimate size, trends, distribution, and habitat use of ungulate populations (Bennett et al. 1940, Rogers et al. 1958, Neff 1968 [comprehensive review], Baily and Putman 1981, Kirchhoff and Pitcher 1988, Koster and Hart 1988, Patterson and Power 2002). In many cases, PG counts were used because ungulate populations inhabited densely forested environments and were difficult to monitor using other techniques requiring direct observation or live capture (Putman 1984, Ratcliffe 1987, Forsyth et al. 2007, van Vliet et al. 2008). Despite wide use and a few rigorous evaluations, several factors limit the utility of PG counts as indices of population trends. These include human error (e.g., pellet detectability, observer experience), variation in pellet deposition rates and pellet persistence (e.g., influence of weather, insects), and the lack of uniformity in pellet-group distribution (Neff 1968, Jenkins and Manly 2008). Moreover, in many circumstances, procedures to convert pellet counts to numbers of animals are based on few empirical data, seldom evaluated over time, and precision associated with estimates rarely quantified. Despite limitations, PG counts were the primary tool for monitoring deer population trends of deer in Southeast Alaska for over 30 years (Kirchhoff and Pitcher 1988, Kirchhoff and Pitcher 1990). That method provided crude estimates of deer abundance, activity really, that were sufficient to detect large changes in populations only. Researchers and managers would benefit from the development of more precise and reliable methods with which to monitor deer populations. During 2004–2009, we designed and tested an alternative approach on Prince of Wales Island, Alaska, which used DNA extracted from fecal pellets to estimate deer population size (Fig 1). Our pilot study provided the first estimates of abundance (based on individually identified deer) for Sitka black-tailed deer, and the first estimates of abundance of a free-ranging ungulate population using DNA from fecal pellets. As a further test of our methods under different population and environmental conditions, the Alaska Department of Fish and Game applied the DNA-based approach on Chichagof Island, Alaska (Fig. 1) during spring 2010. This guide is based mostly on what we learned on Prince of Wales, but also draws on experiences from the Chichagof Island study. Details concerning development of our protocols described in this guide can be found in Brinkman (2009), Brinkman and Hundertmark (2009) and Brinkman et al. (2009, 2010a, 2010b, 2010 [In prep], 2011[In press], Appendix A). Many aspects of this guide will continue to evolve with technology (e.g., genotyping), while others (e.g., transect layout) will certainly improve with further research. Therefore, we will periodically update this document as we learn more and improve methods.

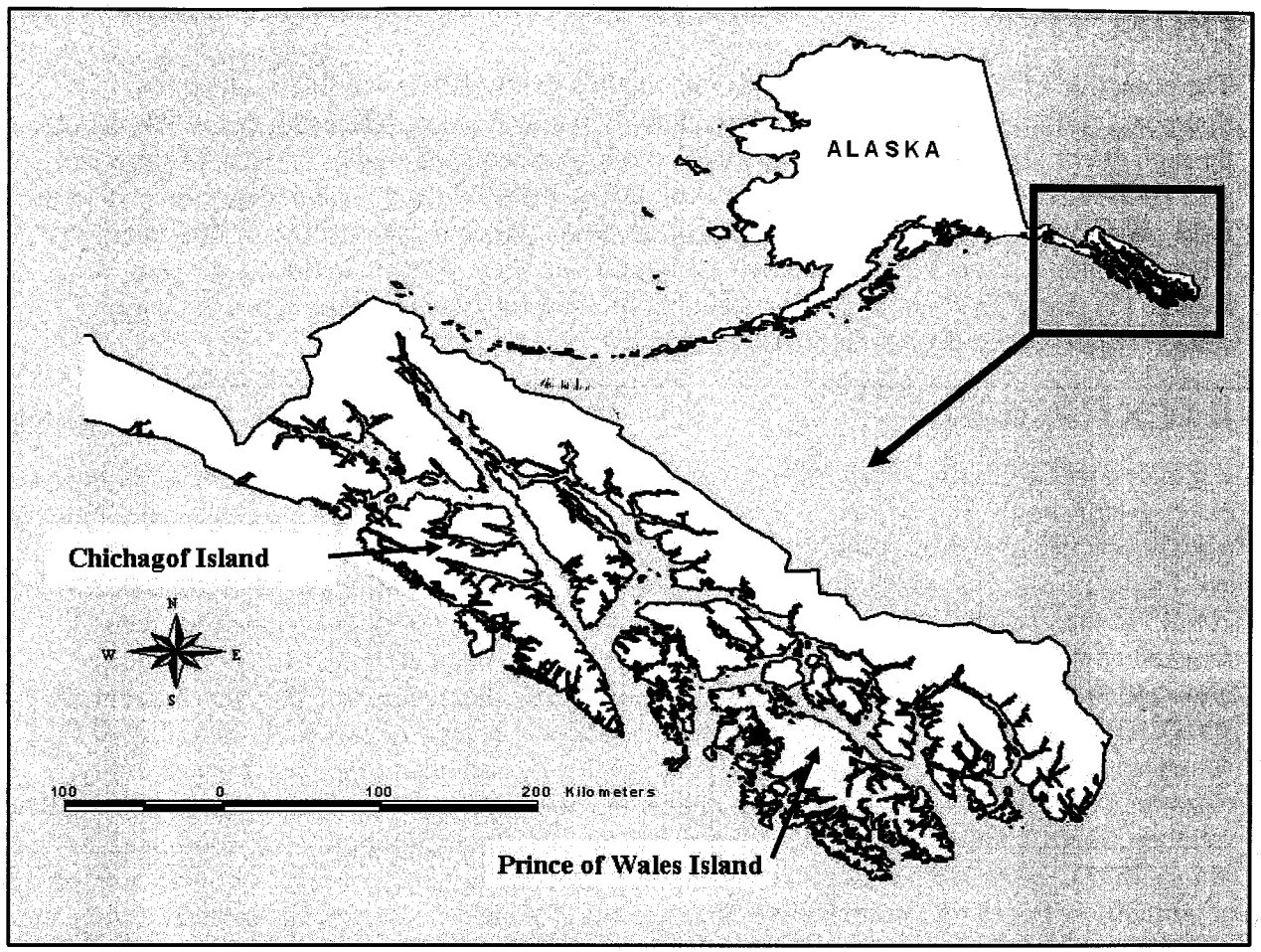

Figure 1. Map of Prince of Wales Island and Chichagof Island in Southeast Alaska.

PELLET SAMPLING PROTOCOL AND ANALYSES

We describe our protocol in 3 parts: sampling design, DNA analyses, and population abundance estimation. In the sampling design section, we provide guidelines for sampling at small and large spatial scales, and designing and implementing path transect sampling. The DNA analyses section provides information on primers, DNA extraction, PCR amplification, genotyping, and error checking. Finally, in population abundance estimation, we review statistical methods employing genotypes to estimate abundance and density, and discuss issues related to inference from the data.

SAMPLING DESIGN

Path Sampling. —Conventional pellet group surveys in Southeast Alaska involve counting pellet groups within 20 m × 1 m linear plots located along straight-line transects starting from permanently marked locations and traversing established bearings. Transects are located within habitats consider to be winter range for deer (productive old-growth forest on southerly aspects below 460 m elevation) and traverse elevations from sea level to 460 m (1500 ft) (Kirchhoff and Pitcher 1988). Our DNA-based approach also establishes and surveys transects, however,

transects follow deer trails rather than straight lines along a single bearing. Trail transects have several advantages over traditional straight-line transects, including: increased encounter rates with pellet groups (50% greater than straight-line transects), applicability in all habitat types, better pellet-detection rates, easier travel through thickly-vegetated habitats, and greater repeatability. Experimenting with 6–8 100m^2 box plots in 4 different watersheds, Brinkman (2009) estimated that deer trails (when buffered by 1 meter on each side of the center of the trail) covered approximately 30% of the area within each plot, and deer deposited 67% of their pellets on the buffered trail network. Fundamentally, the trail transect focuses sampling along trails where activity of deer is greater compared to randomly located straight-line transects; therefore allowing more opportunities to encounter pellet groups, extract DNA from pellets, and identify a higher percentage of the deer in the study area. Moreover, based on simulation modeling, we are concerned that sampling along straight lines may not be capable of even detecting the presence of deer if density is <3.8 deer/km^2 (10 deer/mi^2), whereas that is not likely a limitation with path sampling. Trail transects can be established in all major habitat types: productive old-growth forest, unproductive forests on hydric soils, open muskeg heaths, and clearcut forest at various successional stages (logged 5–60 years ago) under different land management strategies (e.g., precommercially thinned).

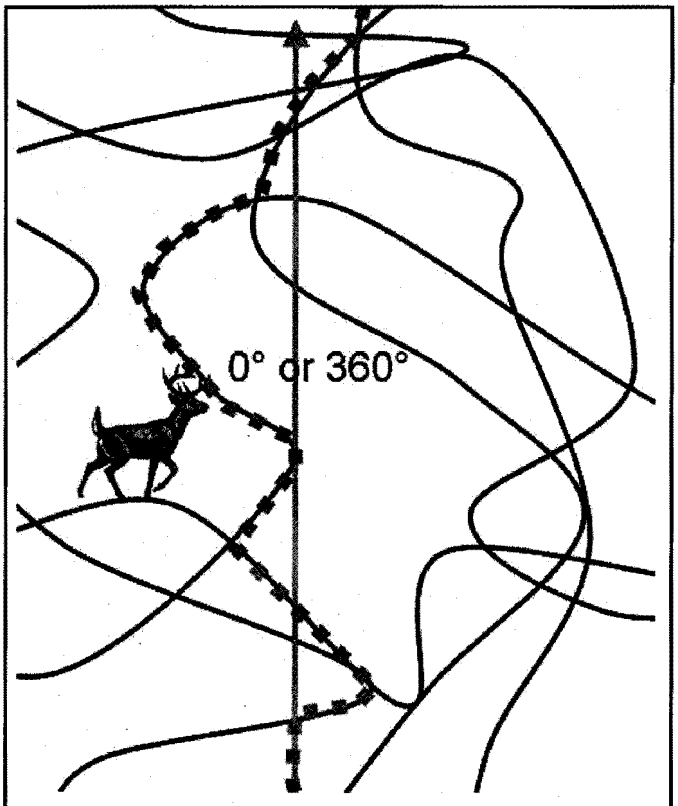

Figure 2. Hypothetical illustration of a trail-transect path relative to traditional straight-line transects.

Path sampling requires a starting point and a bearing (Fig. 2). Trail transects travel in the direction of the chosen bearing from the starting point until a deer trail is encountered. The deer trail is followed until another deer trail intersects the trail being surveyed. If another trail is encountered, a compass is used to determine which trail more closely parallels the direction of the transect bearing and the path transect then follows that trail. If the trail ends or a trail can no longer be identified, the rule is to follow a straight-line path along the bearing direction until another deer trail is encountered. Because of the ubiquity and high density of deer trails in Southeast Alaska, there are frequent opportunities to "self correct" direction of travel toward the initial bearing. During our pilot study (Brinkman et al. 2011, we found that the end point of a trail transect is usually within 50 m of the end point of a straight-line transect. This was evident on transects ranging from 200 m to 2 km long. Using a pre-determined compass bearing to select trails to be surveyed is the

fundamental aspect of the deer-trail technique that minimizes subjectivity of trail selection. Further, systematic selection of deer trails using compass bearings creates an opportunity to objectively sample deer trails that vary with respect to the frequency of deer use.

During establishment, trail transects are intensively marked with fluorescent flagging to ensure repeatability within and across field seasons. Flagging density depends on thickness of vegetation around transects and how often the transect changes deer trails. During our pilot study, flagging trails every 5 meters was sufficient. We recommend being liberal with flagging to allow easier detectability during subsequent surveys. For example, we went through a standard roll of flagging every 300–500 meters of transect. Orange flagging seems be most visible throughout the sampling period. Unfortunately, we can't recommend the use of biodegradable flagging because we found that deer like to browse it. We used a hand-held GPS unit (Garmin xx) to record the location of pellets collected and to track the length of each path transect. Alternatively, hip chains can be used to measure path transect length.

Transect layout.—Transect layout depends on research objectives; however, transect density (i.e., sampling intensity) and positioning (e.g., distance between transects) need careful consideration. During our pilot study (Brinkman et al. 2011), we positioned transects to ensure they traversed a proportionally representative sample of all types of deer habitat that were available in our study sites. For example, if 25% of the landscape in our study site was composed of clearcut forest, then 25% of the total area of transects in that study site traversed was clearcut forest. However, we sampled along individual transects located throughout watersheds. That design limited our ability to convert abundance to density because transects essentially were 2-dimensional paths. To estimate the width representing the area within which deer were likely to be sampled along transects, we estimated maximum mean recapture distance (MMRD) for each deer identified on a transect. The maximum distance separating pellets from the same deer along a transect was calculated for all deer identified and the average value of those distances was then used to define the effective sampling width of each path transect. It is preferable to have transects traversing terrain in perpendicular directions within an area in which animals identified on transects are likely to be resampled (Fig. 3A). On Chichagof Island we experimented with a "node" sampling approach, which should greatly improve density estimation. In node sampling, points or nodes are selected within watersheds that serve as origins of 4 or more path transects that extend in multiple directions (Fig. 3B). Nonetheless, consideration still must be given to the number of nodes and their location with respect to habitat and landscape heterogeneity. Of most importance is the fact that if habitats are sampled proportionally to their occurrence, differences in density of trails between habitats becomes irrelevant, otherwise those differences may introduce bias into estimating abundance and density.

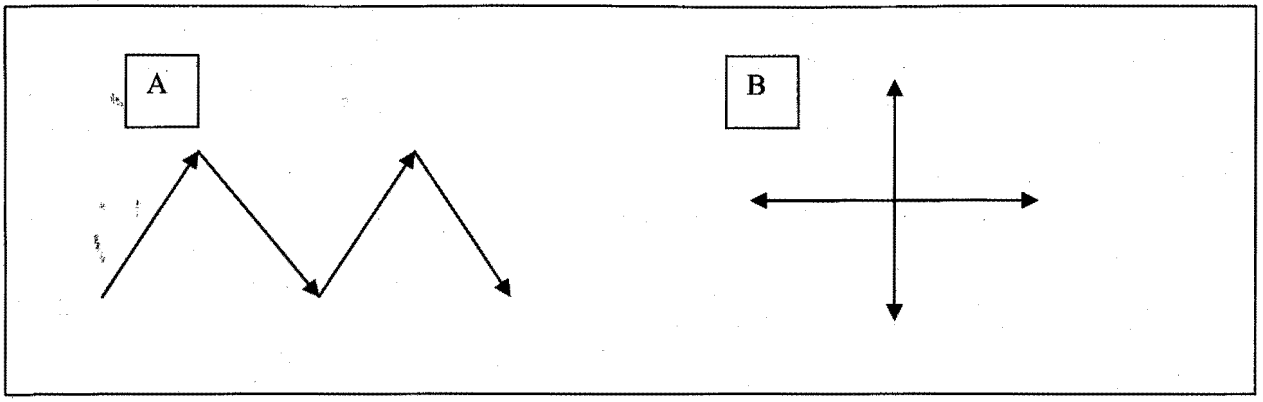

Figure 3. Strategies for laying out path sampling transects that facilitate estimation of animal density. In both examples, path transects traverse ground in perpendicular directions enabling estimation of the area in which deer identified on transects are likely to be resampled.

Spatial intensity of sampling.— Brinkman et al. (2010) sampled a total of approximately 150 m of path transects per km^2 of suitable winter range for deer and achieved abundance estimates with 90% confidence intervals ≤20% of the estimated mean abundance. Suitable range included all habitats used by deer during winter regardless of snow depth. Thus clearcuts, muskegs, and nonproductive forest were included. Essentially the only habitats excluded were alpine, rock, ice, and open water. That strategy is critical to making sampling robust to differing levels of winter severity, a factor that confounds inference from the conventional pellet surveys used in Southeast Alaska. During our efforts on Chichagof Island, we increased sampling intensity to 600 mof total transect length per km^2 of suitable winter deer range. We are still analyzing those data to estimate abundance but the confidence interval should be <20% of the mean. At present we recommend sampling intensity at least within the range of the 2 studies. Moreover, currently our protocol is best applied to studies and monitoring efforts within a single or small group of watersheds. For example, it would be useful for monitoring deer within 4 or 5 watersheds that are popular subsistence hunting areas. The main limitation is the need to resurvey transects multiple times during the sampling period. Nonetheless, we are experimenting with a 1-sample strategy (described below) that only requires a single sampling effort for each node or group of transects within a watershed. Successful application of that strategy would enable our methods to be used at large scales sufficient to monitor region wide populations of deer.

Sampling schedule. — The ideal time to sample transects is in late winter and early spring between snow melt and leaf out when the pellet groups are most visible and factors promoting DNA degradation are lowest (Maudet et al. 2004, Buchan et al. 2005, Murphy et al. 2007, Brinkman et al. 2011). During a typical winter in Southeast Alaska, snow depths condense deer populations in areas below 300m. If transects are surveyed before leaf out and snow melt at higher elevations, it may be possible to assume population closure which can simplify analysis. An assumption of closure is reasonable if deer are not entering or leaving the study area (e.g., migrating, dispersing, fawning, or being killed by hunters or predators). Timing of snow melt mainly determines the number of sampling occasions possible before leaf out. Sampling after leaf out is not recommended because: 1) deer begin spring migration, 2) increase insect and microbial activity degrades pellets at a faster rate after deposition, 3) a shift in the deer's diet can temporarily change the consistency of the feces from pellets to a single runny pile which is both difficult to collect and extract DNA from, and 4) pellets are less detectable. For example, a crew

member from the Chichagof study estimated that pellet detectability was reduced from 2 m on either side of a path transect during the 1st sampling occasion (start of leaf out) to <0.5 m by the 3rd sampling occasion (approximately 20 days later) because of new growth of vegetation.

Although traditional straight-line transects were surveyed once a year (Kirchhoff and Pitcher 1988). Trail transects are designed to be surveyed multiple times (i.e., multiple sampling occasions) each year. This created the opportunity to collect DNA from the same deer multiple times (i.e., recapture) during an annual field season, which is required for mark/recapture analyses (more in *Analysis* section). Brinkman et al. (2011) surveyed individual trails an average of 6.2 (SE = 0.27) times (i.e., sampling occasions) per annual field season. Brinkman et al. (2010a) determined that transects should be resurveyed after an interval of about 10–14 days to ensure that most pellets would yield usable DNA. A narrower window between sampling may increase the freshness of pellets encountered, but a shorter interval may not give deer enough time to return and deposit more pellets on the trail transect. During each sampling occasion, all unsampled pellets are cleared from the transect or pulverized by stomping until pellets are not recognizable or able to sampled during subsequent sampling occasions. This allowed the assumption that all pellet groups encountered during the next sampling occasion were deposited within the last 10 days.

We are experimenting with a single-sample approach using the pellets and subsequent genotype data obtained during the Chichagof study. We will consider each path transect radiating from a sample node to be an individual replicate or resampling event. For example, a node with 4 transects will be treated as having 4 resampling sessions, all surveyed on the same day rather than after 10–14 day intervals. We will examine the precision of abundance estimates derived from those samples and compare it with the same estimates derived from data obtained by resampling the same transects every 10–14 days. If the precision is acceptable, surveying watersheds can be done once during a season and mark-recapture techniques applied to estimate abundance. That would enable our protocol to be used for monitoring deer over large geographic areas because the limitation of having to resurvey transects multiple times during a season is removed.

Pellet sample handling and storage.—We collected 4–6 pellets from each pellet group deposited within 1 m of the center of the deer-trail transect; thus, we were sampling a prescribed width of 2 m (e.g., belt transect [Seber 1982]). Using the protocol in Brinkman et al. (2010b), 1–2 pellets are used during DNA extraction. Therefore, 4–6 pellets allow multiple extractions to be performed if necessary. We recommend selecting pellets from the group that are more protected from the weather. For instance, pellets suspended in the middle of the pile are ideal because the mucus lining containing the deer's DNA is less likely to be washed off by rain or rubbed off or contaminated by ground litter. Although we sampled from pellet groups within the 2-m width to ensure easy detection, all pellets within a 4-m width were removed during each sampling occasion to reduce the chance of sampling from pellet groups that were present during previous sampling occasions. Although conventional straight-line transects surveyed 0.5 meters on each side of the transect, the greater visibility on trail transects allowed a wider detection area.

During collection, pellet samples can be classified based on appearance as: good, average, or poor. "Good" pellets are intact, have a smooth surface with a glossy sheen, have a detectable layer of mucus on the exterior, and are slippery to the touch. A wet and old pellet may still be

shiny and smooth, but only a freshly deposited pellet will be slippery to the touch. "Average" pellet samples were collected from what appeared to be a slightly older or more weathered pellet group which still had intact individual pellets with smooth surfaces, but lacked a tightly clumped distribution, glossy sheen, mucus layer, and are typically more "tacky" to the touch compared to fresh pellets. "Poor" pellet samples were collected from spread-out groups with rough-surfaced pellets which were often showing signs of decomposition.

"Good" samples were twice as likely to yield sufficient DNA to genotype deer compared with "poor" samples. Hence, visual high-grading is possible and may save lab costs. However, such high-grading usually isn't necessary after the 1st sampling occasion because all samples should appear "good" unless they were deposited in standing water or exposed to direct sunlight for several days. During circumstances where sunlight can quickly dry out pellet groups and give an older appearance, we recommend rolling pellets over before classification. Another thing to keep in mind during collection, pellets deposited during late winter tend to be smaller, harder, and darker compared to pellets deposited during early spring. This is because deer are on a more woody diet during the winter months. These pellets can persist much longer in the field and, to an untrained eye, appear fresher than they actually are.

Using a handheld Global Positioning System (GPS), we recorded time and location of each pellet group from which we sampled. Each sample of pellets was collected with unused and sterile latex gloves, placed in plastic conical tubes, filled with 95% ethanol for preservation, and stored at room temperature until DNA extraction.

Labor.— We suggest that a 2-person crew for establishing trail transects. The lead team member uses the compass to select the deer trail to be surveyed, while the second team member follows close behind, searching for pellet groups. Four eyes and feet are better than 2 during the initially sampling occasion with regards to detecting and adequately clearing overwinter deposition of pellets. However, subsequent surveys along the same transects can be done by 1 person. The time needed to survey trail transects will depend on habitat type, pellet density, and topography. A trail transect that travels up steep terrain through thick slash and blow downs with high numbers of pellet groups will take much longer than a flat transect across the middle of a muskeg with low numbers of pellet groups to sample and clear.

During the 2010 study on Chichagof Island, a 2-person crew could establish and sample 3–4 500-m trail transects in 8 hours. Therefore, 1 km of transect per day per person was realistic. However, it is important to point out that during this study and the initial pilot study on POW, all pellet groups that were encountered were counted and their locations recorded using a GPS unit, regardless of whether they were sampled. We did this to create an opportunity to compare DNA-based methods with traditional pellet counts. Because of this approach, crew members spent a significant amount of time entering data into the GPS. Although we haven't quantified this, we speculate that 5–6 500-m transects could be established and sampled in an 8-hour day by a 2-person crew if locations of nonsampled pellet groups were not entered into the GPS.

After the transects are marked and over-winter deposition of pellets cleared, it is possible for subsequent sampling occasions to be performed by 1 person (if bear safety doesn't warrant 2 people). During subsequent sampling occasions, 6–8 500-m transects is possible per person per day, or 2.5–3.0 km of transect. However, both transect layout and seasonal condition will

influence efficiency. For instance, if transect layout requires backtracking between transects, then distance traveled each day will be far greater than distance of transect sampled. We recommend that researchers experiment with transect layouts that optimize efficiency while in the field (Fig. 3). During the pilot study, the final sampling occasion ended with leaf out. During the Chichagof study, the first sampling occasion coincided with leaf out. The difference in labor costs was very evident because of the difference in seasonality. Therefore, we likely overestimated the labor needed based on our pilot studies.

ANALYSIS

During our previous studies, we performed genetic analysis ourselves. However, because of the technical nature of the genetic component of this approach, we understand that biologists may contract a wildlife genetics laboratory to extract DNA from samples, perform PCR reactions, and genotype individual deer. In this guide, our goal is to provide enough information to allow the field biologist to effectively communicate with a genetics laboratory. Details of our DNA extraction, amplification, and genotyping methods are described in Maudet et al. (2004), Brinkman and Hundertmark (2009), and Brinkman et al. (2010a, b). Brinkman and Hundertmark (2009) and Brinkman et al (2010a,b) are included in Appendix A.

DNA extraction.— We recommend transferring the samples to the genetics lab immediately after the end of the field season. During the pilot study, we found that our ability to extract high quality DNA declined over time, even when preserved in 95% ethanol. Ideally, we recommend that DNA-extraction occurs within 3 months of sample collection. Using the DNA extraction protocol established by Brinkman et al. (2010a, b, [Appendix A]), it is important to note that the outside of the deer pellet is what is important, not the inside. When the pellet passes through the lower digestive tract of the deer, epithelial (i.e., tissue) cells from the deer coat the pellets. Essentially, we washed off the lining of the pellet and extracted DNA from the pellet-wash solution. We extracted genomic DNA using the DNeasy Tissue Kit (Qiagen Inc. Valencia, CA).

PCR amplification.— Thirty microsatellite primers have been screened for variability and suitability for use with DNA extracted from Sitka black-tailed deer pellets (Brinkman et al. 2010a). Twenty-six markers (87%) amplified, 20 (67%) were variable, and 7 (23%) amplified consistently with error rates <20% and fit well into a single multiplex; thus, we included those 7 loci in analysis of individual identification. Multiplex PCR reactions contained adjusted concentrations of each primer set to achieve optimum allelic peaks and minimize amplification noise and stuttering. "Multiplex" is when all markers are included in the same PCR reaction, instead of one marker at a time. This saves the lab time and money, which saves the Project a lot of money. We conducted PCR reactions using Qiagen Multiplex Master Mix® (Qiagen, Valencia, CA) according to manufacturer's instructions with slight modifications according to Brinkman et al. (2010b).

Error checking.— Because deer were never observed or handled, muscle, blood or other tissue sample references were not available to compare with DNA extracted from fecal pellets. Therefore, we recommend following a rigorous genotyping protocol to prevent, mitigate, and report genotyping errors. We used a "multi-tube" approach (Taberlet et al. 1996) to identify a consensus genotype and limit errors before statistical modeling. Our estimated probability of identity (PID) calculated using GenAlEx (Peakall and Smouse 2006) was 0.0003 (Brinkman et al

2010b). In general, PID should be <0.001 (Schwartz and Monfort 2008). Summarized by individual marker per reaction, error rates did not exceed 5% during our pilot study. Samples were re-analyzed 3–6 times until a consensus genotype without errors was identified. Through our rigorous genotyping protocol, we discarded 49% of samples and 77% of the 30 microsatellite markers tested to ensure accuracy. During the first year of our pilot study, we successfully genotyped 44% of the samples we collected. By 2008, our genotype success was improved to 87% and likely was influenced by modification of extraction protocol and pellet collection (few "poor" pellets included in analysis).

ABUNDANCE ESTIMATION

You should receive genetic IDs for each sample submitted to the genetic laboratory. Using the spatial and temporal information associated with each ID, mark-recapture analysis can be performed. Each deer genotyped is a "marked" animal and every subsequent re-identification of that animal is a "recapture" event. Data from our pilot study was entered into Program MARK (White and Burnham 1999, White 2008). We used Huggins closed models (Huggins 1991), but also experimented with Open Robust Design models (Kendall and Bjorkland 2001). We evaluated our assumption of population closure using Program CloseTest, which tests the null hypothesis of a closed population model with time variation against the open-population Jolly-Seber model as a specific alternative (Stanley and Burnham 1999). Describing the use of program MARK and its derivatives is beyond the scope of this manual. Model selection is complicated and requires the user to understand model selection techniques and mark-recapture analyses.

Rather than incorporating genotyping error into our statistical models for abundance estimation, we excluded samples and genetic loci that showed signs of error. The cost of this approach was that we lost information when we discarded samples with some degree of error in their genotype. It may be beneficial to test the performance of misidentification models (Lukacs and Burnham 2005). Misidentification models address uncertainty associated with including samples and genetic markers with some degree of genotyping error. That approach may increase sample size and reduce costs associated with re-analyzing the same sample several times to reach a consensus genotype. Other viable approaches for accounting for genotype uncertainty also exist (e.g., Miller et al. 2002, Wright et al. 2009).

Density estimation.— In general, conversion of abundance estimates to density estimates may be biased due to boundary effects that vary with transect layout and home range size (Efford et al. 2004). As stated earlier, locations of our sampling transects did not allow for density to be calculated using maximum likelihood or inverse prediction methods (program DENSITY [Efford et al. 2004, http://www.otago.ac.nz/density]). Brinkman et al. (2011) sampled transects were located irregularly within study sites with regards to spacing and density. We did this to allow representative sampling of all habitat types, but as stated previously, density estimation was compromised because each location was sampled by a single transect that was a narrow band rather than sampling in multiple directions from a common point. Node sampling should eliminate that limitation enabling the use of standard density estimation programs such as DENSITY.

OTHER APPLICATIONS

We also used DNA extracted from pellets to determine sex (Brinkman and Hundertmark 2009). We are currently working on incorporating the genetic marker for sex determination into the muliplex PCR reaction. If successful, this will allow sex identification during the individual identification process at very small increase in lab cost while increasing Probability of Identity. It also will enable biologists to monitor sex ratios within populations and study sex-biased dispersal of deer between watersheds. For example, we observed a strongly female-biased sex ratio for deer within the 3 watersheds on Prince of Wales Island surveyed by Brinkman et al (2011). In addition, a study of deer reproduction and recruitment within the same watersheds indicated dates of parturition for deer spanning June–September (Person and Gilbert unpublished data). Does giving birth late in summer may suggest that breeding is occurring over a protracted period in autumn? That might happen if adult males are few owing to intense harvest, a possibility given support by the strongly skewed sex ratio. In addition, non-invasive genetic sampling creates opportunities to simultaneously address other research questions relating to social structure, paternity, kinship, gene flow, and phylogeography (Kohn and Wayne 1997) of deer in Southeast Alaska. Genetic technology will continue to advance and laboratory costs likely will be reduced. Therefore, we recommend that researchers continue to test study designs (e.g., logistics, flexibility to changing environments, statistical inference) that utilize the rapidly expanding field of wildlife genetics.

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Prince of Wales Island Study

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Chichagof Island Study

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APPENDIX A. PUBLICATIONS DESCRIBING METHODS

Brinkman, T. J., and K. J. Hundertmark. 2009. Sex identification of northern ungulates using low quality and quantity DNA. Conservation Genetics 10:1189-1193.

Brinkman, T. J., D. K. Person, F. Stuart Chapin, III, W. Smith, and K. Hundertmark. 2011. Estimating abundance of Sitka black-tailed deer using DNA from fecal pellets. Journal of Wildlife Management. 75: *In press.*

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