The Journal of Wildlife Management 84(3):492–504; 2020; DOI: 10.1002/jwmg.21820

Research Article

Combining Harvest and Genetics to Estimate Reproduction in Wolves

HEATHER R. CLENDENIN, *Department of Biological Sciences, Institute for Bioinformatics and Evolutionary Studies, University of Idaho, 875 Perimeter Drive MS3051, Moscow, ID 83844-3051, USA*

JENNIFER R. ADAMS, *Department of Fish and Wildlife Sciences, Laboratory for Ecological, Evolutionary and Conservation Genetics, University of Idaho, 875 Perimeter Drive MS1136, Moscow, ID 83844-1136, USA*

DAVID E. AUSBAND,[1] *Idaho Department of Fish and Game, Coeur d'Alene, ID 83815, USA*

JAMES A. HAYDEN, *Idaho Department of Fish and Game, P.O. Box 25, Boise, ID 83814, USA*

PAUL A. HOHENLOHE, *Department of Biological Sciences, Institute for Bioinformatics and Evolutionary Studies, University of Idaho, 875 Perimeter Drive MS3051, Moscow, ID 83844-3051, USA*

LISETTE P. WAITS,[2] *Department of Fish and Wildlife Sciences, University of Idaho, 875 Perimeter Drive MS1136, Moscow, ID 83844-1136, USA*

ABSTRACT Parameters of reproductive success are important to the management of wildlife populations. Genetic monitoring can be an effective approach for acquiring this important demographic information when traditional methods are unsuccessful, inefficient, or too expensive. This study demonstrates a novel application of genetic data opportunistically collected from harvested game to estimate a minimum annual count of breeding packs of gray wolves (*Canis lupus*) and to provide a coarse index of harvest vulnerability of young of the year (YOY) across packs. We used 18 microsatellite loci to genotype 98 gray wolf YOY from 2014 and 105 from 2015 harvested in Idaho, USA. Using this genotype data, we reconstructed sibling groups for each cohort using the program COLONY and treated full-sibling litters as proxies for unique packs. In addition to evaluating our marker panel using simulations, we assessed the accuracy of empirical relationship assignments by adding YOY of known relationship from long-term study packs to the dataset (27 individuals from 2014 and 61 from 2015) and tracking correctly reconstructed relationships. We varied COLONY input parameters to evaluate the power of relationship assignments under conditions that may be encountered when working with empirical data. We also compared COLONY's estimates of effective number of breeders based on sibship frequency to estimates based on a commonly used linkage-disequilibrium method. All COLONY runs for both cohorts correctly identified the known sibling relationships. Among the other individuals, changes in the geographic clustering of putative siblings, probabilities of inclusion and exclusion for reconstructed sibling groups, and consistency of relationship assignments across COLONY runs suggested that marker number had a larger effect on accuracy than access to population-level genetic data. Our estimates of breeding packs subjected to harvest within the state (52 for 2014 and 63 for 2015) differed from estimates reported by Idaho Department of Fish and Game by ≤6 for both years. Among packs that had pups harvested, most packs had 1–2 YOY harvested, whereas other packs had as many as 5 YOY harvested. All estimates of the number of effective breeders had overlapping confidence intervals regardless of method, though sibship frequency-based estimates had larger confidence intervals than estimates using the linkage disequilibrium method. Our study shows that sibling relationships can be accurately and reliably reconstructed from harvested gray wolves, and demonstrates a valuable new use of samples collected through harvest. © 2020 The Wildlife Society.

KEY WORDS *Canis lupus*, genetic monitoring, harvest, reproduction, sibship reconstruction, western United States.

Monitoring demographic parameters of populations is an important and demanding component of adaptive management. Comprehensive and cost-effective monitoring using traditional methods, such as direct observation and radio-telemetry, can be intractable for species that are remotely located, behaviorally elusive, cryptic, or distributed across large geographic ranges (Ausband et al. 2014). Under these circumstances, genetic monitoring can be an efficient approach to obtaining reliable demographic information (De Barba et al. 2010, Stansbury et al. 2014). Genetic data can help identify species and individuals, provide estimates of population parameters, and offer insights into space use and connectivity (Schwartz et al. 2007, Paetkau et al. 2009, Mumma et al. 2015, Micheletti and Storfer 2017).

Received: 26 February 2019; Accepted: 25 November 2019

[1]*Current affiliation: U.S. Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho, 875 Perimeter Drive MS1141, Moscow, ID 83844-1141, USA*
[2]*E-mail: lwaits@uidaho.edu*

Measures of relatedness and genetic diversity can be used to reconstruct pedigrees, gain greater understanding of mating systems, assess population viability, and track quantitative traits (Thomas and Hill 2000, DeWoody 2005, Lucia and Keane 2011, Putnam and Ivy 2014, Gooley et al. 2017).

Relatedness analyses and kinship assignments compare genetic data from groups of individuals and use assumptions of identity by descent and Mendelian inheritance to assign relatedness within the context of relationship categories or as a continuous measure of genetic similarity (DeWoody 2005). These measures can also be used to track movement and reproduction, estimate census and effective population sizes, and allow for close-kin genetic mark-recapture (Tokarska et al. 2009, Hauser et al. 2011, Fabbri et al. 2012, Artiles et al. 2015, Yu et al. 2015).

Sibship reconstruction is a relatedness assignment method that attempts to identify individuals of the same cohort that belong to common sibling groups (Almudevar and Anderson 2012). Likelihood methods are commonly applied in the inference of sibships without parental information (Painter 1997, Wang 2004, Ashley et al. 2008). The program COLONY is a maximum likelihood-based method that uses a group-wise method to evaluate the likelihood of entire pedigree configurations and has been found to be a powerful and accurate approach to relationship reconstruction (Walling et al. 2010, Hauser et al. 2011, Karaket and Poompuang 2012, Ackerman et al. 2017). The certainty and reliability of sibship reconstruction methods are influenced by the number of genetic markers used and their variability within a population (DeWoody 2005). Using insufficiently informative markers can reduce the power of discernment and result in incorrect assignments, and failing to account for population allele frequencies can bias inferences through the disproportionate representation of alleles present in sampled families (Wang 2012). Adequate data collection is fundamental to accurate and reliable sibship assignments; however, resources available for monitoring and management of wild populations are often limited and careful consideration must be given to their allocation.

The management of gray wolves (*Canis lupus*) within Idaho, USA, demonstrates such efforts to maximize the return on resources invested in monitoring. Gray wolves are managed as a game species in Idaho and are also monitored to ensure the population remains above recovery levels (Stansbury et al. 2014, Idaho Department of Fish and Game [IDFG] 2016). Responses to harvest within the conterminous United States vary from those documented in populations farther north, making ongoing observation vital to responsive management actions (Ausband 2016). Although federal funding to support wolf monitoring has declined following delisting and the state no longer uses a radio-telemetry-based approach for monitoring, less intensive monitoring using carefully selected, complementary methods have been effective (Stenglein et al. 2011, Ausband et al. 2014).

Existing studies have addressed questions relevant to wolf population ecology through direct observation, telemetry, hunter surveys, non-invasive genetic monitoring, and models of occupancy and vital rates (Rich et al. 2013, Bassing et al. 2015, Hindrikson et al. 2017, Stansbury et al. 2016, Granroth-Wilding et al. 2017). Taking advantage of harvest reporting and ongoing genetic monitoring, we propose use of sibship reconstruction as a novel method for estimating a minimum count of reproductively active wolf packs. Although sibship assignment requires more markers than individual identification, few additional resources are needed to add these analyses to the existing genetic monitoring program. Tissue samples and premolars are collected by IDFG personnel during mandatory harvest reporting, which facilitates genotyping and aging of harvested individuals. The number of litters affected by harvest can be found by reconstructing sibships among harvested young of the year, which can be treated as a proxy for a minimum count of reproductively successful packs. Reconstructing sibgroups (i.e., full-sibling litters) among harvested young of the year can also be used as a coarse index of harvest vulnerability across packs; for instance, the effect of harvesting several young of the year within a relatively small area could vary if these individuals belonged to separate packs or if they represented a large portion of a single pack's mean reproductive output.

In this project, we sought to assess the feasibility of using sibship reconstruction of harvested young of the year as a method for estimating a minimum count of reproductively successful wolf packs within Idaho and compared estimates of the effective number of breeders (N_b) based on sibship frequency to another common single-sample N_b estimator. We hypothesized that the most accurately and reliably reconstructed sibling configurations would come from runs using the longest run times and highest likelihood precision, and from the treatment using the full marker set and background allele frequency data, and that the number of markers would have a larger bearing on the robustness of assignments than the use of background population allele frequency data.

STUDY AREA

Idaho (216,632 km^2) contains many different landscapes, including mountainous forests, desert shrub, prairies, and open valleys. Elevations in the state range from 217 m to >3,859 m. Public forests and private timber holdings, dominated by western red cedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), and Douglas-fir (*Pseudotsuga menziesii*) comprised most areas in northern Idaho. Management zones in central Idaho contained a mixture of wilderness areas, native prairies, and private agricultural land, whereas areas in southern Idaho were predominantly private agricultural land (Mack et al. 2010). Annual precipitation ranged from <20 cm to >250 cm, with temperatures ranging from −34°C to 38°C (Western Regional Climate Center 2010). Gray wolves in 2013 and 2017 occurred and were subject to harvest throughout the state, with higher abundances in the northern and central portions. Hunting and trapping were regulated across 13 wolf

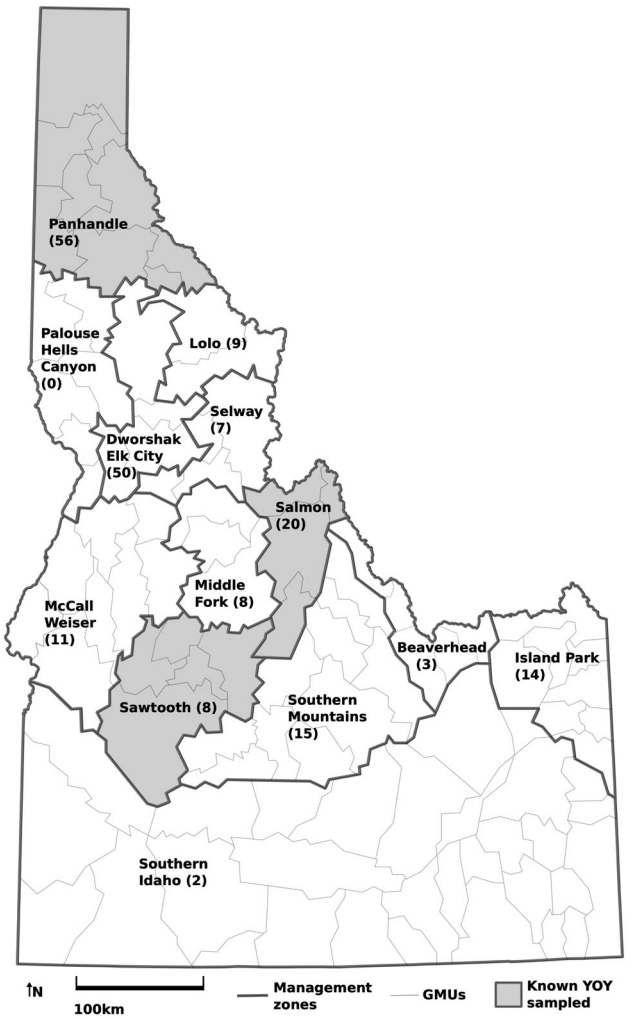

Figure 1. Spatial distribution of harvested gray wolf young of the year (YOY) from 2014 and 2015, as reported within the 13 wolf management zones in Idaho, USA. Numbers in parentheses following the wolf management zone names indicate the total count of harvested YOY used in sibling reconstructions across both years. Individuals of known relationship category were sampled in the shaded management zones. These individuals were not included in the depicted counts but included 23 YOY from the Panhandle wolf management zone, 34 from Salmon, and 31 from Sawtooth. Game management units (GMUs) are also depicted by fine lines within wolf management zones.

management zones, subdivided into 98 game management units (Fig. 1).

METHODS

Sampling

Personnel from IDFG collected tissue samples from voucher specimens provided during harvest reporting in 12 of Idaho's 13 wolf management zones (WMZs) during 2014 and 2015 (IDFG and Nez Perce Tribe 2014, IDFG 2015). Reporting of wolf harvest in Idaho is mandatory, first via a telephone hotline and second by bringing, at a minimum, the hide and skull of harvested wolves to IDFG personnel. Personnel from IDFG record location of harvest, means of take, animal condition, and date of harvest and affix a pelt tag to the animal. Among the harvested wolves, we identified cohorts of young of the year (YOY) for 2014 and 2015 using cementum analysis of premolars extracted during reporting (Matson's Laboratory, Manhattan, MT, USA). We used 98 YOY from 2014 and 105 from 2015 in sibship analyses (Fig. 1).

We embedded individuals of known relationship (i.e., both siblings and non-siblings) within the dataset of harvested YOY to assess rates of correct assignment and the consistency of these assignments. The YOY of known relationship came from both cohorts and were sampled from long-term study packs within 3 of the management zones within the state (27 from 2014; 61 from 2015; Fig. 1). Individuals within these packs have been monitored through annual field surveys and fecal DNA sampling at rendezvous sites, allowing for individual identification, pack assignment, and pedigree reconstruction (Stenglein et al. 2010, Ausband et al. 2014, Stansbury et al. 2016). We followed University of Montana animal use protocols (001-15MMMCWRU-011315 and 008-12MMMWCRU-021412) during surveys. Four YOY/year from long-term study packs were harvested and are also represented among the harvested YOY counts.

Additionally, we used genotype data from wolves documented through Idaho's long-term genetic monitoring program to estimate background population-level allele frequencies and locus error rates (i.e., allelic drop-out, mistyping rates, and other errors). These estimates were based on 18-locus genotypes of tissue samples from 865 wolves collected in Idaho between September 2013 and September 2017 using the maximum-likelihood estimate reliability method of Miller et al. (2002).

Genotyping

We extracted DNA from 20-mg samples of tissue using Qiagen DNeasy Blood and Tissue kits, with negative controls included to test for contamination (Qiagen, Valencia, CA, USA). We combined 18 dye-labelled nuclear DNA microsatellite loci into 2 polymerase chain reaction (PCR) multiplexes with a product size of <300 base pairs (AHT103, AHT109, AHT121, AHT200, C05.377, C09.173, C37.172, Cxx.119, Cxx.250, FH2001, FH2004, FH2010, FH2054, FH2088, FH2137, FH2611, FH2670, FH3725; Holmes et al. 1994, Breen et al. 2001, Guyon et al. 2003, Salim et al. 2007, Ostrander et al. 2017). We ran each multiplex PCR with a negative control to test for possible contamination of reagents.

The 7-uL PCR reactions for both multiplexes contained 3.5 uL of 1.5× concentrated Qiagen Master Mix, 0.7 uL of 0.5× concentrated Qiagen Q_Solution, and 2 uL DNA extract (for details see Tables S1–S4, available online in the Supporting Information). We separated PCR products using an Applied Biosystems 3130xl capillary machine (Applied Biosystems, Foster City, CA, USA), and scored genotypes with GENEMAPPER 5.0 (Applied Biosystems). We ran samples in duplicate and repeated the process a third time when necessary to resolve genotype inconsistencies or to address failure due to sample preservation quality. We used samples with consensus genotypes

(i.e., alleles independently identified at least twice) at 90% or more of the loci in sibship reconstruction (Fig. 1). We coded non-consensus genotypes as missing data.

Marker Evaluation

The full marker set of 18 loci are used in a long-term gray wolf genetic monitoring program within Idaho (Stenglein et al. 2011, Ausband et al. 2014, Stansbury et al. 2016). These loci were developed as 2 multiplexes. The first multiplex includes 10 loci and is the standard for individual identification of noninvasively collected samples. The second multiplex adds 8 additional loci. The full set of 18 loci allows for assignment of parentage and identification of family groups. We used GENALEX to calculate the allelic richness, observed heterozygosity, and expected heterozygosity for the complete 18-loci panel (Table S5, available online in Supporting Information; Peakall and Smouse 2012).

We used several methods to evaluate the full marker set's power to determine relationships. We used GENALEX to calculate the probability of identity (P_{ID}) and the more rigorous probability of identity based on siblings ($P_{ID(sibs)}$; Waits et al. 2001). We assessed the power to discern between full-sibling and half-sibling relationships based on the 18-locus set using the analytical method in KININFOR (Wang 2006).

We also simulated pedigrees in COLONY with the same marker characteristics to assess power to accurately assign relationships. These simulated pedigrees were based on generated genotypes using the same number of loci with the same allele numbers, allele frequencies, and error rates as our empirical marker set. We ran these simulations under 2 alternate scenarios: 1 in which all relationships were either full-siblings or unrelated singleton YOY, and another in which half-sibling relationships were also included. The number of YOY simulated corresponded to each respective cohort and the number of parents corresponded to estimates from our empirical reconstructions. The full-sibling scenarios imitated the family size and structure of our empirical results, and approximately half of the corresponding groups in the half-sibling scenario included a range of half-sibling members. We ran full-sibling reconstructions under the same parameters as our empirical datasets, save for run length (short) and likelihood precision (low). We evaluated accuracy of assignment by relationship category for 10 replicates of each scenario.

Empirical Sibship Reconstruction

We separately reconstructed full-sibling relationships for each cohort using the software COLONY 2 (Jones and Wang 2010). In the interest of extending these methods to other systems where monitoring resources may differ, we ran sibship reconstructions with various permutations of marker numbers and background information. To assess sensitivity of COLONY sibship assignments to input and parameter settings, we created 3 treatments (Table 1). Treatment 18BD (i.e., 18 microsatellite loci with background data) used population allele frequencies and error rate estimates calculated from 865 genotyped wolves from Idaho's long-term genetic monitoring program. Treatment 18NBD (i.e., 18 microsatellite loci with no background data) was also based on 18 loci, but allele frequencies and error rates were inferred only from individuals sampled within a given cohort. Treatment 10BD (i.e., 10 microsatellite loci with background data) used a subset of 10 loci and incorporated background population data into allele frequency and error rate estimates.

Because of the nature of the optimization algorithm employed by COLONY, reconstructions based on insufficiently informative markers may not necessarily converge on the same configuration of full-sibling families (Jones and Wang 2010). Inconsistent and inaccurate assignments are more likely to occur when run length is shortened, either through adjusting the run-length parameter or by reducing the likelihood precision. As such, we used tracking changes in configuration likelihood and other measures of support while implementing a series of runs typical of parameter optimization, including both replicates and a range of parameter settings, to reveal differences in power among treatments. We varied parameter settings across 5 sets of COLONY runs for each treatment (Table 1). We evaluated correct identification of known

Table 1. Parameter settings in the software COLONY used for sibship reconstruction of gray wolf young of the year harvested in 2014 and 2015 in Idaho, USA. The parameters altered between treatments (i.e., 18 loci with background data [18BD], 18 loci without background data [18NBD], and 10 loci with background data [10BD]) and we included the method used to calculate allele frequencies and the number of loci analyzed.

Treatment	Run	Run length	Full-likelihood precision	Random number seed	Allele frequencies	Number of loci
18BD	1	Short	Low	Default	Known	18
	2	Short	High	Default	Known	18
	3	Short	High	Altered	Known	18
	4	Medium	High	Default	Known	18
	5	High	High	Default	Known	18
18NBD	1	Short	Low	Default	Unknown	18
	2	Short	High	Default	Unknown	18
	3	Short	High	Altered	Unknown	18
	4	Medium	High	Default	Unknown	18
	5	High	High	Default	Unknown	18
10BD	1	Short	Low	Default	Known	10
	2	Short	High	Default	Known	10
	3	Short	High	Altered	Known	10
	4	Medium	High	Default	Known	10
	5	High	High	Default	Known	10

relationships based on accurate assignment of individuals from long-term study packs, total number of sibgroups, counts of members within sibgroups, and consistency of assigned relationships between individuals for differences between runs and treatments.

Assessment of Sibship Assignments

The inclusion of YOY from long-term study packs (27 from 2014; 61 from 2015; Fig. 1) provided *a priori* knowledge of relationship categories. Individuals within the same pack were known to be siblings, whereas individuals in different packs were known to be non-siblings. These individuals comprised 6 known sibling groups in the 2014 cohort and 13 known sibling groups in the 2015 cohort. In subsequently reconstructed sibgroups, these known relationships allowed us to identify incorrectly included or excluded individuals within this subset.

Relationships that do not remain consistent across COLONY runs may indicate inaccurate assignments. We tracked inconsistent sibship assignments within each treatment to assess the reliability of reconstructed relationships. For each possible pair of siblings within a cohort, we evaluated relationship assignments within treatments by comparing the respective results of each COLONY run and identifying changes in assigned relationship categories. For example, if a given sibgroup contained members A, B, and C, the sibling pairs would be A-B, A-C, and B-C; an inconsistent assignment could exclude member C, affecting pairs A-C and B-C, whereas an inconsistent assignment adding member D to the sibling group would include the additional sibling pairs A-D, B-D, and C-D. We scored all possible pairings per COLONY run as either siblings (1) or non-siblings (0), with these scores summed across all 5 runs. Pairs of individuals with relationship assignments that remained consistent across all runs had summed scores of either 5 (always assigned as siblings) or 0 (never assigned as siblings), with intermediate scores indicating inconsistent assignments across COLONY runs within a treatment.

COLONY reports probabilities of inclusion (i.e., the probability that a full-sibling family contains only true siblings) and exclusion (i.e., the probability that a putative family contains true full-siblings and no true siblings have been incorrectly excluded) for each putative sibling group. We can expect a family containing only 1 individual to always have an inclusion probability of 1 and expect the exclusion probability to always be equal to or less than the inclusion probability (Jones and Wang 2010). We evaluated inclusion and exclusion probabilities for sibgroups across treatment types for statistically significant differences using Kruskal-Wallis rank sum tests and Wilcoxon rank sum tests in Program R, version 3.4.0 (R Core Team 2017).

As an additional assessment of the plausibility of sibling assignments, we compared the game management units (GMUs) and WMZs associated with the reporting of each harvested individual for each member of a putative sibgroup. Management delineations do not necessarily correspond to territories or home ranges; however, geographical clustering can be expected of true siblings and disparate geographical locations of putative siblings may signal inaccurate assignments or incorrect reporting. Although reported territories vary in size between 33 km^2 to 4,335 km^2, typical dispersal distances average around 96.3 km (Boyd and Pletscher 1999, Jimenez et al. 2017). Though some exceptional distances have been recorded, both pre-dispersal forays and long-term dispersal typically occur at no earlier than 11 months of age, making long-distance travel unlikely among the YOY included in our analyses (Boyd and Pletscher 1999, Mech and Boitani 2003, Vilà et al. 2003, Jimenez et al. 2017). We categorized reconstructed sibgroups with ≥2 detected members by those with all members associated with the same GMU, all members associated with the same WMZ but not within the same GMU, all members within adjacent WMZs, or members distributed in some other manner (Fig. 1). We generated counts and percentages of the evaluated sibgroups within each category, and averaged categories across runs for each treatment for sibling groups that did not contain consistent individual relationship assignments.

We compared the number of reconstructed sibling groups, as a proxy for reproductive packs, for each cohort to the estimated minimum number of reproductive packs based on field observations as reported annually by IDFG (IDFG and Nez Perce Tribe 2014, IDFG 2015). We also compared these values at a finer geographical scale by contrasting the number of reconstructed sibling groups to the field estimate of reproductive packs within a WMZ for each year. We treated the count of sibgroups with all members detected within a given WMZ as a minimum count for the sibship reconstruction method, adding sibling groups with any members within a given WMZ as a maximum, and compared this range of values to the field estimates reported by IDFG.

Estimate of Effective Number of Breeders

COLONY assesses sibship frequency to produce estimates of effective population size (N_e) for populations with discrete generations or N_b for populations with overlapping generations. COLONY's approach combines genetic and demographic parameters to generate this estimate (Wang 2009). We compiled these estimates generated using the full 18 loci and background data, treatment 18BD, for each cohort along with 95% confidence intervals.

To provide an external point of reference, we also estimated N_b based on genetic parameters with the linkage-disequilibrium (LD) method implemented in N_EESTIMATOR (Do et al. 2014). We used the same full 18-locus genotypes for each cohort to generate LD-based estimates of N_b. We selected the monogamy model and set a minor allele frequency (MAF) threshold of 0.01.

RESULTS

Marker Evaluation

The set of 18 microsatellite loci had enough power to discern between closely related individuals ($P_{ID} = 3.69 \times 10^{-20}$ and $P_{ID(sibs)} = 5.28 \times 10^{-8}$). Power to discern specifically

between full-sibling and half-sibling relationships was also high (0.74).

Reconstructed sibling groups based on the simulated full-sibling pedigrees correctly identified all relationships across 10 replicates even with short runs and low likelihood precision, which is expected to have lower accuracy than the respective parameters used in our empirical sibship reconstructions (Jones and Wang 2010). Reconstructions with the same parameter settings for the more complex pedigrees containing half-siblings were also highly accurate. All full-siblings were correctly identified across the 10 replicates simulating the 2014 cohort, whereas we observed 1 occurrence of a misidentified full-sibling across the 10 replicates representing the 2015 cohort. Half-siblings were accurately identified 93.4% of the time across the 10 replicates representing the 2014 cohort and 95.2% of the time for the replicates representing the 2015 cohort. Misidentified half-siblings were consistently incorrectly assigned as non-siblings with 1 exception (i.e., incorrectly assigned as full-siblings) in 2014. Non-siblings were correctly identified more than 99.9% of the time for both years.

Number of Litters by Treatment

Among the 2014 cohort, there were no changes in the number of assigned sibling groups across runs in treatments 18BD and 18NBD, though estimates varied between treatments (52 and 53, respectively). The number of litters (46 and 47) were not consistent across runs in treatment 10BD. In the 2015 cohort, all runs of treatments 18BD and 10BD generated the same number of litters (63 and 55 litters, respectively), and estimates in all but 1 18NBD run were the same (runs 1, 2, 3, and 5 all had 64 sibling groups, and run 4 had 63; Fig. 2).

For both cohorts, fewer sibgroups were reconstructed in treatments using 10-locus genotypes relative to the full 18-locus panel. Most of this reduction can be attributed to smaller sibling groups identified in treatments 18BD and 18NBD being added to larger families and singletons being paired together. In the 2014 cohort, 13 individuals designated as singletons in treatments 18BD and 18NBD were joined into pairs or absorbed into larger groups in treatment 10BD, and 3 pairs were added to larger groups. In the 2015 cohort, 12 singletons and 11 pairs assigned in treatments 18BD and 18NBD were assigned to larger groups in treatment 10BD (Fig. 2; Table 2).

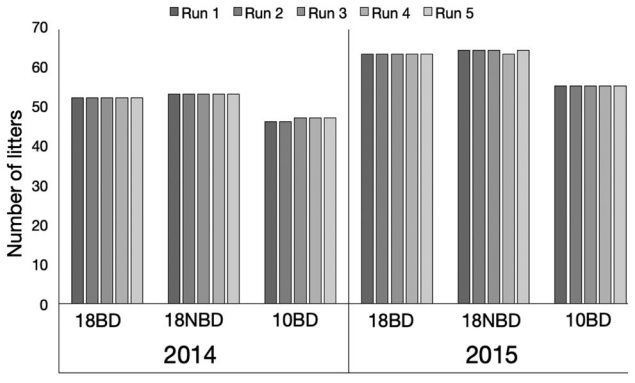

Figure 2. Estimated counts of gray wolf litters assigned by cohort year, treatment type (i.e., 18 loci with background data [18BD], 18 loci without background data [18NBD], 10 loci with background data [10BD]), and run number. Estimates are based on the number of putative sibling groups reconstructed by COLONY among young of the year harvested in Idaho, USA, 2014–2015.

Table 2. Mean count of putative litters by group size across COLONY runs and all treatment types for gray wolf young of the year (YOY) harvested in Idaho, USA, in 2014 and 2015. Litters of YOY of known relationship are not included. Treatments included 18 loci with background data (18BD), 18 loci without background data (18NBD), and 10 loci with background data (10BD).

		Number of group members				
Year	Treatment	1	2	3	4	5
2014	18BD	14.0	20.0	8.0	4.0	0.0
	18NBD	6.2	21.4	9.0	2.0	2.0
	10BD	20.2	17.4	8.8	4.4	0.0
2015	18BD	16.2	18.8	8.0	3.8	0.2
	18NBD	18.0	20.0	8.0	3.0	1.0
	10BD	10.0	13.0	14.0	5.0	0.0

Assessment of Sibship Assignments

Across all runs and treatments, all individuals of known relationship from the long-term study packs were correctly categorized, falling within 6 known sibling groups in the 2014 cohort and 13 known sibling groups in the 2015 cohort. No individuals known to be from separate packs were incorrectly assigned as siblings; however, 2 harvested individuals from the 2015 cohort that had not been reported among the long-term study packs were assigned to sibgroups with known-relationship wolves across all runs, with an additional harvested individual included under treatment 10BD. The full 18-locus microsatellite genotypes of these harvested individuals were compatible with their putative siblings (i.e., all alleles had previously been observed within this sibgroup), and subsequent parentage analyses using COLONY and genotype data from long-term genetic monitoring confirmed shared parentage with assigned littermates for the 2 harvested individuals included among known sibgroups across all treatments (D. E. Ausband, IDFG, unpublished data). However, parentage analysis indicated that although the harvested individual added to a known sibgroup under treatment 10BD could have shared maternity with its putative siblings, it appeared to have been sired by an unrelated male (vonHoldt et al. 2007, Ausband 2018). Although the parentage analysis was not an independent evaluation of pack membership, it did provide additional support suggesting ≥2 harvested individuals had true membership in long-term study packs despite lack of previous detection.

Reconstructions for the 2014 cohort had 1 treatment without any rearranged full-sibling pairs across the 5 runs (18BD). Reconstructions for the 2015 cohort had 2 treatments without any full-sibling rearrangements across runs (18BD and 10BD). Overall, the number of consistently assigned full-sibling pairs was greater than the number of rearranged pairs across runs and treatments (Table 3).

Our analyses indicated that there were significant differences in the inclusion and exclusion probabilities across

Table 3. Rearrangement of putative full-sibling pairs by treatment and cohort for gray wolf young of the year harvested in Idaho, USA, in 2014 and 2015. Litter count describes the mean total number of reconstructed sibling groups assigned within a given cohort and treatment (i.e., 18 loci with background data [18BD], 18 loci without background data [18NBD], and 10 loci with background data [10BD]). Observed pairs specifies unique individual sibling pairings within assigned sibling groups. Rearranged pairs describes the number of individual sibling pairings observed in at ≥1 configuration that were not consistent across COLONY runs within the same treatment.

Year	Treatment	Litter count ($\bar{x}$)	Observed pairs	Rearranged pairs	Rearranged pair frequency across 5 runs
2014	18BD	52.0	126	0	Not applicable
	18NBD	53.0	131	9	6 pairs observed in one run; 3 pairs observed in 4 runs
	10BD	46.6	142	7	4 pairs observed in 2 runs; 3 pairs observed in 3 runs
2015	18BD	63.0	205	0	Not applicable
	18NBD	63.8	210	13	1 pair observed in 1 run; 6 pairs observed in 2 runs; 6 pairs observed in 3 runs
	10BD	55.0	222	0	Not applicable

Table 4. Mean probabilities of inclusion and exclusion across all sibling groups of reconstructed configurations within a given treatment (i.e., 18 loci with background data [18BD], 18 loci without background data [18NBD], and 10 loci with background data [10BD]) and cohort of gray wolf young of the year harvested in Idaho, USA, in 2014 and 2015. An asterisk indicates values significantly different from others within the same cohort based on Kruskal-Wallis rank sum tests and Wilcoxon rank sum tests with *P*-values of <0.05.

Year	Treatment	Inclusion	Exclusion
2014	18BD	0.95	0.56
	18NBD	0.97	0.57
	10BD	0.92*	0.40*
2015	18BD	0.97	0.48
	18NBD	0.97	0.49
	10BD	0.96*	0.38*

treatments (Kruskal-Wallis $\chi^2_2 = 8.84$, $P = 0.01$ for inclusion probabilities in 2014 and Kruskal-Wallis $\chi^2_2 = 23.96$, $P < 0.01$ in 2015; Kruskal-Wallis $\chi^2_2 = 30.56$, $P < 0.01$ for exclusion probabilities in 2014 and Kruskal-Wallis $\chi^2_2 = 12.58$, $P < 0.01$ in 2015). Specifically, the treatment with significantly different mean probabilities of inclusion and exclusion in both cohorts was 10BD (Table 4). Wilcoxon rank sum tests indicated that both the mean inclusion and exclusion probabilities for treatment 10BD were significantly lower than the other treatments in the 2014 cohort and the 2015 cohort (Table S6, available online in Supporting Information). We did not detect significant differences between 18BD and 18NBD in either cohort (Table S6, Figs. S1 and S2, available online in Supporting Information).

Treatments 18BD and 18NBD performed similarly with respect to the spatial distribution of individuals assigned as siblings, with most putative sibling groups in both cohorts consisting of members all within the same GMU or within the same WMZ. Treatment 10BD had the smallest fraction of sibgroups with members detected in the same management unit across cohorts and showed the greatest disparity in spatial distribution of putative siblings (Fig. 3).

Detection of reproductive packs at the state-level was comparable between established field methods and sibship reconstruction (Fig. 4; Table S7, available online in Supporting Information). For 2014, IDFG reported an estimate of 55 reproductive packs minimum, and sibship reconstruction detected 52 when using the full set of 18 loci and background population data. Similarly for 2015, IDFG reported 69 reproductive packs minimum and sibship reconstruction detected 63.

At the WMZ level, many estimated minimum counts of reproductive packs based on field observations fell within

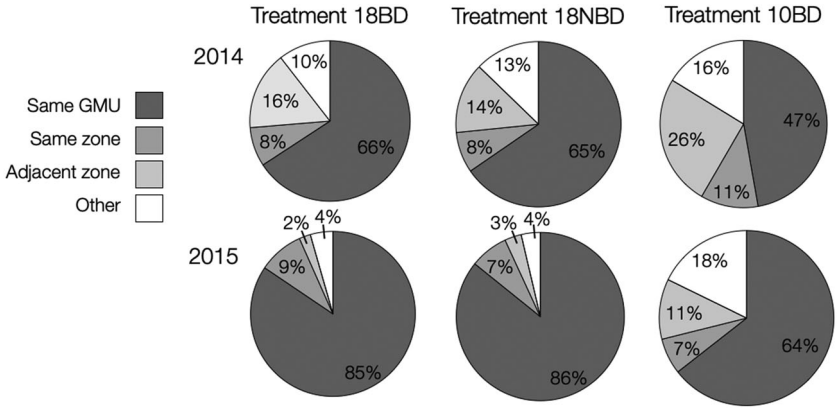

Figure 3. For sibling groups of harvested gray wolf young of the year (YOY) with ≥2 members detected in 2014 and 2015 within Idaho, USA, the percent of groups across cohorts and treatments (i.e., 18 loci with background data [18BD], 18 loci without background data [18NBD], and 10 loci with background data [10BD]) that fell under each spatial distribution category is depicted by the pie graphs. Same game management unit (GMU) entails all members were reported in the same GMU within the same wolf management zone. Same zone depicts groups in which all members were within the same wolf management zone, but ≥1 member was not within the same GMU. Groups categorized under adjacent zone contain ≥1 member that was not in the same wolf management zone as other putative siblings, but all members were reported to wolf management zones with shared borders. Groups categorized as other contained ≥1 member that was in non-adjacent wolf management zone relative to other putative siblings.

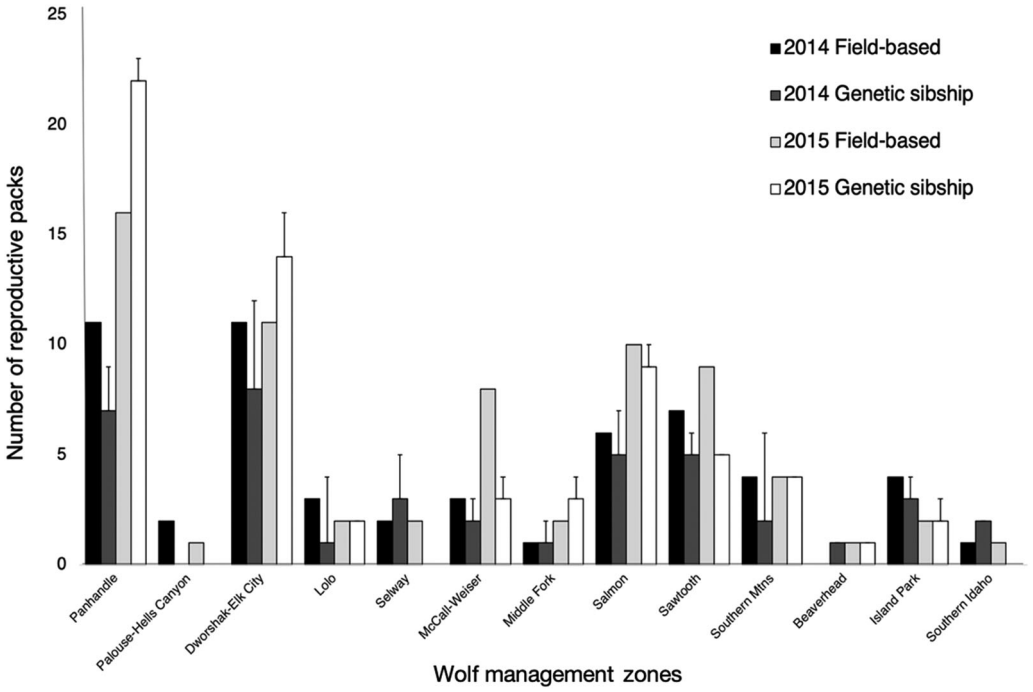

Figure 4. The estimated minimum counts of reproductive gray wolf packs by wolf management zone (WMZ) in Idaho, USA, 2014–2015, as reported by the Idaho Department of Fish and Game (IDFG) using field methods, and as determined through sibship reconstruction. Field-based values correspond to estimated minimum counts documented in IDFG annual harvest reports. Genetic sibship values represent the number of sibling groups estimated from genetic analysis with all members reported within the same WMZ. The total possible number of sibships per WMZ based on all sibling groups with any member in a given WMZ are represented by error bars.

the range of values estimated using sibship reconstruction or were otherwise comparable (Fig. 4). We observed the largest differences between field estimates and the estimates based on sibship reconstruction in the Panhandle WMZ. This zone had the largest numbers of reproductive packs detected overall (11 based on field methods and 7–9 based on sibship reconstruction in 2014, and 16 based on field methods and 22–23 based on sibship reconstruction in 2015).

Estimate of Effective Number of Breeders

The estimates of effective number of breeders calculated in COLONY using sibship frequency for the 2014 cohort ranged from 117 using a random mating model to 102 using a non-random mating model. In 2015, the respective estimates ranged from 120 (random mating) to 96 (non-random mating). The LD method implemented in N_EESTIMATOR produced an estimate of 120.3 in 2014 and 102.4 in 2015. All estimates had overlapping 95% confidence intervals across both years, though these intervals were smaller for the LD-based estimates relative to the sibship frequency-based estimates (Fig. 5).

DISCUSSION

Our study shows that sibling relationships can be accurately and reliably reconstructed from harvest samples using the program COLONY. Our work assists with the management of a terrestrial game species and demonstrates a valuable new use of samples collected through harvest. Genetic sibship reconstruction of harvested YOY can augment existing monitoring methods by providing a minimum count of reproductive wolf packs, an estimate of the effective number of breeders, and a coarse index of harvest vulnerability of young across packs (e.g., harvest was a source of mortality for 1–2 pups in most affected packs, but as many as 5 YOY were harvested from others; Table 2). Although actual litter sizes for reproductive wolf packs cannot be determined using genetic sibship reconstruction of harvested young, it can be used within the context of mean litter size to gauge the overall harvest pressure across packs. We also found that sibship frequency-based estimates of the effective number of breeders were consistent across sample years and had overlapping confidence intervals with estimates based on linkage disequilibrium, another commonly employed single-sample N_b estimator. The value of estimating counts of parents and family groups through sibship reconstruction has been demonstrated in other systems, such as monitoring abundance of social bee colonies by identifying sisters among foraging workers and estimating the number of female sea turtles (green [*Chelonia mydas*] and Kemp's ridley [*Lepidochelys kempii*] sea turtles) laying multiple clutches per season at the same nesting site (Toquenaga and Kokuvo 2010; Frey et al. 2013, 2014; Geib et al. 2015). Fisheries and aquaculture systems have used similar methods to shed light on genetic variability between age stages, assess stocking strategies, and reconstruct putative parental genotypes among externally fertilized species (Liu and Ely 2009, Li et al. 2013, Meraner et al. 2013, Hasanat et al. 2014). These applications, like ours, expand the information gained through genetic monitoring approaches.

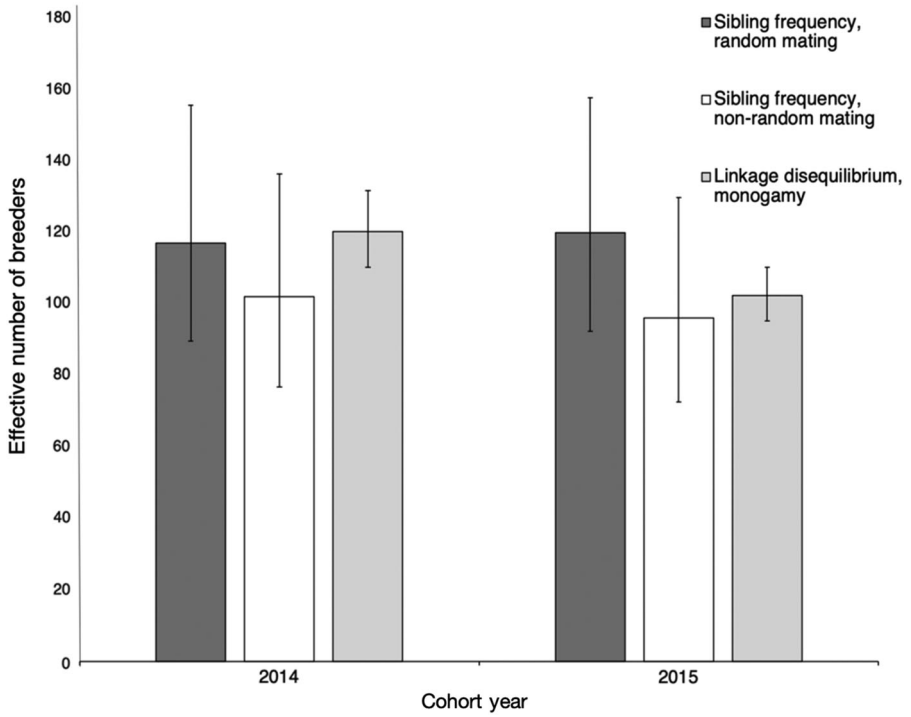

Figure 5. Estimates of the effective number of breeders for the 2014 and 2015 cohorts of gray wolf young of the year in Idaho, USA. We estimated the sibship frequency method, both under the random mating and non-random mating models, using COLONY. We estimated the linkage disequilibrium method using N_EESTIMATOR with a minor-allele frequency threshold of 0.01. Error bars depict 95% confidence intervals.

Integrating multiple sources of information on wildlife populations can help to circumvent some of the challenges and weaknesses of individual monitoring methods and better capture demographic trends and responses to management actions (Ausband et al. 2014, Horne et al. 2019). In our system, with an existing genetic monitoring program, sibship reconstruction requires few additional resources to estimate a minimum count of breeding packs within Idaho. The recovery status of wolves in the state was contingent upon maintaining a minimum number of breeding pairs (i.e., 10 pairs in either Idaho or Montana annually, or <15 pairs in either state for 3 consecutive years; U.S. Fish and Wildlife Service [USFWS] 2011). The 2009 USFWS Wolf Delisting Rule, however, defines breeding pairs as an adult male and an adult female wolf that have produced ≥2 pups that survived until 31 December of the year of their birth, during the previous breeding season (USFWS 2009). Given these stipulations, an estimate based on sibship reconstruction does not directly meet the formal legal criteria regarding breeding pairs. Nonetheless, this estimate could serve as a reasonable substitute for more resource-intensive methods of obtaining minimum counts of reproductive groups that have been used to validate probabilities of packs containing breeders and calibrate population models (Mitchell et al. 2008, 2010, Gude et al. 2009, Ausband et al. 2014). When assessed with other measures of population size, reproductive rates, and distribution of harvest pressure, this estimate can be treated as an index to track changes in the number of breeding pairs and population size.

In our study, we aimed to identify methods to optimize accuracy and certainty of sibling assignments. Sibship reconstruction accurately and consistently identified all *a priori* known relationships across all treatments and runs. Systematic variation of input parameters allowed us to compare correct identification of known relationships and consistency of sibship reconstructions under conditions that may be encountered with other managed populations, allowing for assessment of parameter sensitivity and robustness of assignments. We found that the number of markers used and the method of calculating allele frequencies had a greater effect on the accuracy and reliability of assignments than changes to the stringency of COLONY parameter settings, such as run length and likelihood precision. Using a reduced set of 10 loci decreased the number of family groups detected and produced less credible individual assignments. When we used all 18 loci, sibship reconstructions identified a greater number of unique litters, with more probable assignments and greater spatial cohesion among putative siblings. Assignments using this full marker set were consistent across all runs when we used background population data to estimate allele frequencies, indicating further refinement and reliability of individual relationships under these conditions. A sampling scheme less representative of the population or including fewer individuals, however, may be less accurate without background allele frequency data.

Although the true number of total sibling groups was not known for either cohort, identification of known relationships and other response variables can be used to guide considerations in the application of sibship reconstruction. Notably, our results suggested that there were inaccurate

individual assignments when using the restricted set of 10 loci. Using insufficient marker data appears to have produced errors of false inclusion, resulting in significantly lower probabilities of inclusion and exclusion, significantly lower total group counts, and a larger percent of sibling groups that contained putative members with less spatial proximity to each other than siblings identified in other treatments (Fig. 3). Erroneous assignments have been observed elsewhere under conditions where, because of insufficient marker information and family structure, non-siblings have genotypes consistent with full-siblings by chance (Chapman et al. 2003, Wang and Santure 2009, Lepais et al. 2010).

In contrast to the reconstructions using 10 loci, the congruency in results across treatments using the full marker set lends assurance that the 18 microsatellite loci panel and the threshold set for missing data provide enough information and power to make these assessments, with or without background allele frequency data. The increased total counts of sibling groups in these treatments likely represent unique reproductive packs, which reconstructions using fewer markers were not able to discriminate. Overall, using the complete marker set and applying existing genetic monitoring data produced assignments in which group counts and individual relationships were the most consistent and reliable.

Our proposed monitoring method of using the number of reconstructed litters from harvested YOY as a proxy for the number of reproductive packs provides only a minimum count and will be limited by harvest rates and distribution. For instance, we cannot ascertain whether differences in estimates across years reflects changes in the number of reproductive packs or is a consequence of sampling differences. The confidence intervals for the estimated number of breeders overlapped between years, however, suggesting that this metric may be less susceptible to differences in sample sizes. Monitoring both minimum counts of reproductive packs along with estimates of the effective number of breeders may provide a more meaningful gauge of population dynamics than either alone.

Other uncertainties and sources of error should be considered when adding sibship reconstruction to population monitoring. Variation in the quality of sample preservation can affect estimates because poorly preserved samples often have lower genotyping success rates and could decrease detection probabilities. Estimates can be further complicated by incidences of extra-pair matings; sneaker males have been documented as sires and multiple breeders have been observed within packs (vonHoldt et al. 2007, Ausband 2018). Sibship reconstruction of harvested YOY also cannot be used to explicitly determine whether a harvested individual originated from breeding packs within the same geographical boundaries in which it was detected. Additionally, the spatial distribution of putative siblings within sibgroups indicated that 10–13% of the sibgroups observed may have contained spurious assignments. COLONY does not use an exclusion-based assignment method and tends to err toward over-joining groups, which can reduce the total number of groups. Although less concerning when estimating a minimum count of reproductive packs than it might be under other circumstances, it is possible that our samples originated from more litters than estimated (Jones and Wang 2010). Despite different sources of error and missing data between the implemented methods, our estimates of minimum reproductive pack counts were close to annual estimates reported by IDFG based on other survey methods, both at the state and WMZ level (Table 5; Fig. 4).

Table 5. Estimates of population parameters for gray wolves in Idaho, USA, in the 2014 and 2015 biological years, including (from top to bottom) minimum count of reproductive packs based on sibling reconstruction and estimates from other Idaho Department of Fish and Game (IDFG) monitoring efforts, estimates of effective number of breeders (N_b) based on sibship frequency (SF) under the random mating and non-random mating models estimated using COLONY and the linkage disequilibrium (LD) method estimated using N_EESTIMATOR with a minor-allele frequency threshold of 0.01, the total number of estimated packs within Idaho, IDFG's estimate of census size, number of wolves harvested each year, the number of harvested young of the year (YOY) genotyped, and the number of YOY used in sibling reconstructions, including individuals sampled from long-term study packs.

Year	2014	2015
Min. count (genetic sibship)	52	63
Min. count (field observation)	55	69
SF N_b (random mating)	117	120
SF N_b (non-random mating)	102	96
LD N_b (monogamy)	120.3	102.4
Estimated packs in ID	104	108
Census estimate (IDFG)	770	786
Harvested wolves	256	256
Harvested YOY genotyped	98	105
YOY analyzed	121	162

Though harvest as a monitoring tool entails different challenges in assessing detection probabilities, there are several advantages to this source of data (Leclerc et al. 2016). Hunter surveys and harvest reporting provide valuable information on wildlife populations and engage stakeholders in the monitoring process (Rich et al. 2013, Leclerc et al. 2016). Additionally, genetic analyses based on harvested individuals can complement non-invasive genetic sampling. Non-invasive sampling is limited by agency time and resources, resulting in patchiness in spatial detection patterns. Though harvest can also exhibit spatial bias, these biases are not likely to be the same, allowing the geographical distribution of harvest-based sampling to supplement that of agency monitoring (Leclerc et al. 2016). Individual assignment and aging of harvested samples can be more accurate than estimation based on non-invasive sampling. Non-invasive genetic samples are generally lower quality and have lower genotyping success than tissue samples. Approximating age based on relative diameter of scat entails subjective interpretation (Weaver and Fritts 1979). In contrast, the teeth of wolves under a year old generally do not have closed roots, making cementum analysis a more explicit means of designating an individual as YOY (E. A. Ausband, personal communication, Matson's Laboratory, Manhattan, MT, USA). Recognizing this valuable resource, sibship reconstruction of harvested

YOY can take advantage of an opportunistic source of data to provide further information on managed populations.

MANAGEMENT IMPLICATIONS

Estimating minimum counts of reproductive packs provides a metric for assessing whether a population managed as a game species is meeting management objectives. Harvest provides a valuable, opportunistic source of samples. Genetic reconstruction of sibling groups from harvest samples is an efficient, reliable way to estimate a minimum count of reproductive packs. Genetic sibship reconstruction also provides a coarse measure of the vulnerability of young to harvest across packs. The existing panel of 18 microsatellites used in Idaho's long-term genetic monitoring program provides sufficient power to assign relationships among our gray wolf population. Application of this method to other systems will be affected by background population knowledge, marker number, allelic richness, and heterozygosity of the markers used. Implementation of genetic reconstruction should be preceded by evaluation of a marker panel's power to assign relationships.

ACKNOWLEDGMENTS

We thank the IDFG field technicians and biologists for collection of samples, and specifically J. L. Struthers for collection, management, and coordination of samples. We thank the Laboratory of Ecological, Evolutionary, and Conservation Genetics for the laboratory support, especially M. M. Keyes, D. S. Gour, and K. L. Cochrell for assistance with genotyping and analyses. We are grateful for the thoughtful feedback from C. R. Miller and from our reviewers. Financial support was provided by IDFG and University of Idaho College of Natural Resources.

LITERATURE CITED

Ackerman, M. W., B. K. Hand, R. K. Waples, G. Luikart, R. S. Waples, C. A. Steele, B. A. Garner, J. McCane, and M. R. Campbell. 2017. Effective number of breeders from sibship reconstruction: empirical evaluations using hatchery steelhead. Evolutionary Applications 10: 146–160.

Almudevar, A., and E. C. Anderson. 2012. A new version of PRT software for sibling groups reconstruction with comments regarding several issues in the sibling reconstruction problem. Molecular Ecology Resources 12:164–178.

Artiles, A., R. Cobo, L. Benítez, L. Pérez, and G. Espinosa. 2015. Assessment of genetic variation and productive markers through four progenies of the first introduced stock of cultured shrimp *Penaeus* (*Litopenaeus*) *vannamei* in Cuba. International Journal of Aquaculture 5(23):1–12.

Ashley, M. V., T. Y. Berger-Wolf, I. C. Caballero, W. Chaovalitwongse, B. DasGupta, and S. I. Sheikh. 2008. Computational biology: new research. Nova Science, Hauppauge, New York, USA.

Ausband, D. E. 2016. Gray wolf harvest in Idaho. Wildlife Society Bulletin 40:500–505.

Ausband, D. E. 2018. Multiple breeding individuals within groups in a social carnivore. Journal of Mammalogy 99:836–844.

Ausband, D. E., L. N. Rich, E. M. Glenn, M. S. Mitchell, P. Zager, D. A. W. Miller, L. P. Waits, B. B. Ackerman, and C. M. Mack. 2014. Monitoring gray wolf populations using multiple survey methods. Journal of Wildlife Management 78:335–346.

Bassing, S. B., M. S. Mitchell, D. E. Ausband, L. P. Waits, and G. Hale. 2015. Developing a monitoring framework to estimate wolf distribution and abundance in Southwest Alberta. Intermountain Journal of Sciences 21:56–57.

Boyd, D. K., and D. H. Pletscher. 1999. Characteristics of dispersal in a colonizing wolf population in the Central Rocky Mountains. Journal of Wildlife Management 63:1094–1108.

Breen, M., S. Jouquand, C. Renier, C. S. Mellersh, C. Hitte, N. G. Holmes, A. Chéron, N. Suter, F. Vignaux, A. E. Bristow, et al. 2001. Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. Genome Research 11:1784–1795.

Chapman, R. E., J. Wang, and A. F. Bourke. 2003. Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators. Molecular Ecology 12:2801–2808.

De Barba, M., L. P. Waits, E. O. Garton, P. Genovesi, E. Randi, A. Mustoni, and C. Groff. 2010. The power of genetic monitoring for studying demography, ecology and genetics of a reintroduced brown bear population. Molecular Ecology 19:3938–3951.

DeWoody, J. A. 2005. Molecular approaches to the study of parentage, relatedness, and fitness: practical applications for wild animals. Journal of Wildlife Management 69:1400–1418.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillet, and J. R. Ovenden. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. Molecular Ecology Resources 14:209–214.

Fabbri, E., R. Caniglia, N. Mucci, H. P. Thomsen, K. Krag, C. Pertoldi, V. Loeschcke, and E. Randi. 2012. Comparison of single nucleotide polymorphisms and microsatellites in non-invasive genetic monitoring of a wolf population. Archives of Biological Sciences 64:321–335.

Frey, A., P. H. Dutton, and G. H. Balazs. 2013. Insights on the demography of cryptic nesting by green turtles (*Chelonia mydas*) in the main Hawaiian islands from genetic relatedness analysis. Journal of Experimental Marine Biology and Ecology 442:80–87.

Frey, A., P. H. Dutton, D. J. Shaver, J. S. Walker, and C. Rubio. 2014. Kemp's Ridley *Lepidochelys kempii* nesting abundance in Texas, USA: a novel approach using genetics to improve population census. Endangered Species Research 23:63–71.

Geib, J. C., J. P. Strange, and C. Galen. 2015. Bumble bee nest abundance, foraging distance, and host-plant reproduction: implications for management and conservation. Ecological Applications 25:768–778.

Gooley, R., C. J. Hogg, K. Belov, and C. E. Grueber. 2017. No evidence of inbreeding depression in a Tasmanian devil insurance population despite significant variation in inbreeding. Scientific Reports 7:1830.

Granroth-Wilding, H., C. Primmer, M. Lindqvist, J. Poutanen, O. Thalmann, J. Aspi, J. Harmoinen, I. Kojola, and T. Laaksonen. 2017. Non-invasive genetic monitoring involving citizen science enables reconstruction of current pack dynamics in a re-establishing wolf population. BMC Ecology 17:44.

Gude, J. A., M. S. Mitchell, D. E. Ausband, C. A. Sime, and E. E. Bangs. 2009. Internal validation of predictive logistic regression models for decision-making in wildlife management. Wildlife Biology 15:352–369.

Guyon, R., T. D. Lorentzen, C. Hitte, L. Kim, E. Cadieu, H. G. Parker, P. Quignon, J. K. Lowe, C. Renier, B. Gelfenbeyn, et al. 2003. A 1-Mb resolution radiation hybrid map of the canine genome. Proceedings of the National Academy of Sciences 100:5296–5301.

Hasanat, A., F. A. Mollah, and S. Alam. 2014. Assessment of genetic diversity in wild and hatchery populations of mrigal *Cirrhinus cirrhosus* (Hamilton-Buchanan) using allozyme markers. International Journal of Fisheries and Aquatic Studies 1(4):24–31.

Hauser, L., M. Baird, R. Hilborn, L. W. Seeb, and J. E. Seeb. 2011. An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon (*Oncorhynchus nerka*) population. Molecular Ecology Resources 11:150–161.

Hindrikson, M., J. Remm, M. Pilot, R. Godinho, A. V. Stronen, L. Baltrūnaité, S. D. Czarnomska, J. A. Leonard, E. Randi, C. Nowak, et al. 2017. Wolf population genetics in Europe: a systematic review, meta-analysis and suggestions for conservation and management. Biological Reviews 92:1601–1629.

Holmes, N. G., N. J. Strange, M. M. Binns, C. S. Mellersh, and J. Sampson. 1994. Three polymorphic canine microsatellites. Animal Genetics 25:200.

Horne, J. S., D. E. Ausband, M. A. Hurley, J. Struthers, J. E. Berg, and K. Groth. 2019. Integrated population model to improve knowledge and management of Idaho wolves. Journal of Wildlife Management 83:32–42.

Idaho Department of Fish and Game [IDFG]. 2015. 2015 Idaho wolf monitoring and progress report. IDFG, Boise, Idaho, USA.
Idaho Department of Fish and Game [IDFG]. 2016. Statewide report surveys and inventory: wolf surveys and inventories, wolf population status and trend studies. IDFG, Boise, Idaho, USA.
Idaho Department of Fish and Game [IDFG] and Nez Perce Tribe. 2014. Idaho wolf monitoring and progress report. IDFG, Boise and Nez Perce Tribe, Lapwai, Idaho, USA.
Jimenez, M. D., E. E. Bangs, D. K. Boyd, D. W. Smith, S. A. Becker, D. E. Ausband, S. P. Woodruff, E. H. Bradley, J. Holyan, and K. Laudon. 2017. Wolf dispersal in the Rocky Mountains, Western United States: 1993–2008. Journal of Wildlife Management 81:581–592.
Jones, O. R., and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551–555.
Karaket, T., and S. Poompuang. 2012. CERVUS vs. COLONY for successful parentage and sibship determinations in freshwater prawn *Macrobrachium rosenbergii* de Man. Aquaculture 324:307–311.
Leclerc, M., J. Van de Walle, A. Zedrosser, J. E. Swenson, and F. Pelletier. 2016. Can hunting data be used to estimate unbiased population parameters? A case study on brown bears. Biology Letters 12:20160197.
Lepais, O., B. Darvill., S. O'Connor, J. L. Osborne, R. A. Sanderson, J. Cussans, L. Goffe, and D. Goulson. 2010. Estimation of bumblebee queen dispersal distances using sibship reconstruction method. Molecular Ecology 19:819–831.
Li, R., Q. Li, and C. Wang. 2013. Sibship reconstruction and effective population size estimation in mass spawning ark shell, *Scapharca broughtonii* based on microsatellite analysis. Genes & Genomics 35:703–708.
Liu, J. X., and B. Ely. 2009. Sibship reconstruction demonstrates the extremely low effective population size of striped bass *Morone saxatilis* in the Santee–Cooper system, South Carolina, USA. Molecular Ecology 18:4112–4120.
Lucia, K. E., and B. Keane. 2011. A field test of the effects of familiarity and relatedness on social associations and reproduction in prairie voles. Behavioral Ecology and Sociobiology 66:13–27.
Mack, C., R. Rachael, J. Hoylan, J. Husseman, M. Lucid, and B. Thomas. 2010. Wolf conservation and management in Idaho; progress report 2009. Nez Perce Tribe Wolf Recovery Project, Lapwai, Idaho. Idaho Department of Fish and Game, Boise, USA.
Mech, L. D., and L. Boitani. 2003. Wolves: behavior, ecology, and conservation. University of Chicago, Chicago, Illinois, USA.
Meraner, A., G. Unfer, and A. Gandolfi. 2013. Good news for conservation: mitochondrial and microsatellite DNA data detect limited genetic signatures of inter-basin fish transfer in *Thymallus thymallus* (Salmonidae) from the Upper Drava River. Knowledge and Management of Aquatic Ecosystems 409:1–17.
Micheletti, S. J., and A. Storfer. 2017. An approach for identifying cryptic barriers to gene flow that limit species' geographic ranges. Molecular Ecology 26:490–504.
Miller, C., P. Joyce, and L. Waits. 2002. Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160:357–366.
Mitchell, M. S., D. E. Ausband, C. A. Sime, E. E. Bangs, J. A. Gude, J. A. Jimenez, C. M. Mack, T. J. Meier, M. S. Nadeau, and D. W. Smith. 2008. Estimation of successful breeding pairs for wolves in the Northern Rocky Mountains, USA. Journal of Wildlife Management 72:881–891.
Mitchell, M. S., J. A. Gude, D. E. Ausband, C. A. Sime, E. E. Bangs, M. D. Jimenez, C. M. Mack, T. J. Meier, M. S. Nadeau, and D. W. Smith. 2010. Temporal validation of an estimator for successful breeding pairs of wolves *Canis lupus* in the U.S. Northern Rocky Mountains. Wildlife Biology 16:101–106.
Mumma, M. A., C. Zieminski, T. K. Fuller, S. P. Mahoney, and L. P. Waits. 2015. Evaluating noninvasive genetic sampling techniques to estimate large carnivore abundance. Molecular Ecology Resources 15:1133–1144.
Ostrander, E. A., R. K. Wayne, A. H. Freedman, and B. W. Davis. 2017. Demographic history, selection and functional diversity of the canine genome. Nature Reviews Genetics 18:705–720.
Paetkau, D., E. Vázquez-Domínguez, N. I. Tucker, and C. Moritz. 2009. Monitoring movement into and through a newly planted rainforest corridor using genetic analysis of natal origin. Ecological Management & Restoration 10:210–216.
Painter, I. 1997. Sibship reconstruction without parental information. Journal of Agricultural, Biological, and Environmental Statistics 2:212–229.
Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539.
Putnam, A. S., and J. A. Ivy. 2014. Kinship-based management strategies for captive breeding programs when pedigrees are unknown or uncertain. Journal of Heredity 105:303–311.
R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Rich, Li. N., R. E. Russell, E. M. Glenn, M. S. Mitchell, J. A. Gude, K. M. Podruzny, C. A. Sime, K. Laudon, D. E. Ausband, and J. D. Nichols. 2013. Estimating occupancy and predicting numbers of gray wolf packs in Montana using hunter surveys. Journal of Wildlife Management 77:1280–1289.
Salim, D. C., A. A. Akimoto, C. B. Carvalho, S. F. Oliveira, C. K. Grisolia, J. R. Moreira, and M. N. Klautau-Guimarães. 2007. Genetic variability in maned wolf based on heterologous short-tandem repeat markers from domestic dog. Genetics and Molecular Research 6:348–357.
Schwartz, M. K., G. Luikart, and R. S. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology & Evolution 22:25–33.
Stansbury, C. R., D. E. Ausband, P. Zager, C. M. Mack, C. R. Miller, M. W. Pennell, and L. P. Waits. 2014. A long-term population monitoring approach for a wide-ranging carnivore: noninvasive genetic sampling of gray wolf rendezvous sites in Idaho, USA. Journal of Wildlife Management 78:1040–1049.
Stansbury, C. R., D. E. Ausband, P. Zager, C. M. Mack, and L. P. Waits. 2016. Identifying gray wolf packs and dispersers using noninvasive genetic samples. Journal of Wildlife Management 80:1408–1419.
Stenglein, J. L., L. P. Waits, D. E. Ausband, P. Zager, and C. M. Mack. 2010. Efficient, noninvasive genetic sampling for monitoring reintroduced wolves. Journal of Wildlife Management 74:1050–1058.
Stenglein, J. L., L. P. Waits, D. E. Ausband, P. Zager, and C. M. Mack. 2011. Estimating gray wolf pack size and family relationships using noninvasive genetic sampling at rendezvous sites. Journal of Mammalogy 92:784–795.
Thomas, S. C., and W. G. Hill. 2000. Estimating quantitative genetic parameters using sibships reconstructed from marker data. Genetics 155:1961–1972.
Tokarska, M., T. Marshall, R. Kowalczyk, J. M. Wójcik, C. Pertoldi, T. N. Kristensen, V. Loeschcke, V. R. Gregersen, and C. Bendixen. 2009. Effectiveness of microsatellite and SNP markers for parentage and identity analysis in species with low genetic diversity: the case of European bison. Heredity 103:326–332.
Toquenaga, Y., and N. Kokuvo. 2010. Full-sib reconstruction in haplodiploid populations. Applied Entomology and Zoology 45:59–64.
U.S. Fish and Wildlife Service [USFWS]. 2009. Endangered and threatened wildlife and plants; final rule to identify the northern Rocky Mountain population of gray wolf as a distinct population segment and to revise the list of Endangered and Threatened wildlife. Federal Register 74:15123–15188.
U.S. Fish and Wildlife Service [USFWS]. 2011. Reissuance of final rule to identify the Northern Rocky Mountain Population of gray wolf as a distinct population segment and to revise the list of endangered and threatened wildlife. Federal Register 76:25590–25592.
Vilà, C., A. K. Sundqvist, Ø. Flagstad, J. Seddon, S. Björnerfeldt, I. Kojola, A. Casulli, H. Sand, P. Wabakken, and H. Ellegren. 2003. Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. Proceedings. Biological Sciences 270(1510):91–97.
vonHoldt, B., D. R. Stahler, D. W. Smith, D. A. Earl, J. P. Pollinger, and R. K. Wayne. 2007. The genealogy and genetic viability of reintroduced Yellowstone grey wolves. Molecular Ecology 17:252–274.
Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10:249–256.
Walling, C. A., J. M. Pemberton, J. D. Hadfield, and L. E. B. Kruuk. 2010. Comparing parentage inference software: reanalysis of a red deer pedigree. Molecular Ecology 19:1914–1928.
Wang, J. 2004. Sibship reconstruction from genetic data with typing errors. Genetics 166:1963–1979.
Wang, J. 2006. Informativeness of genetic markers for pairwise relationship and relatedness inference. Theoretical Population Biology 70:300–321.

Wang, J. 2009. A new method for estimating effective population sizes from a single sample of multilocus genotypes. Molecular Ecology 18:2148–2164.
Wang, J. 2012. Computationally efficient sibship and parentage assignment from multilocus marker data. Genetics 191:183–194.
Wang, J., and A. W. Santure. 2009. Parentage and sibship inference from multilocus genotype data under polygamy. Genetics 181:1579–1594.
Weaver, J., and S. H. Fritts. 1979. Comparison of coyote and wolf scat diameters. Journal of Wildlife Management 43:786–788.
Western Regional Climate Center. 2010. Historical climate information. <http://www.wrcc.dri.edu>. Accessed 12 Nov 2010.
Yu, G. C., Q. Z. Tang, K. R. Long, T. D. Che, M. Z. Li, and S. R. Shuai. 2015. Effectiveness of microsatellite and single nucleotide polymorphism markers for parentage analysis in European domestic pigs. Genetics and Molecular Research 14:1362–1370.

Associate Editor: Zach Olson.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.