

# Evaluating the efficacy of non-invasive genetic methods and estimating wolf survival during a ten-year period

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**Abstract** Genetic capture–recapture (CR) analysis is a highly promising tool to estimate population parameters and monitor populations through time. However, its level of accuracy has rarely been assessed and comparisons with traditional estimates in controlled settings have rarely been performed. We used CR analysis with long-term fecal genotyping data and applied open-population models to estimate survival rates and assess trend in abundance of wolves in Isle Royale over 10 years, while simultaneously estimating those parameters with traditional aerial-based techniques that are believed to be reasonably accurate. Comparison of the techniques indicated that there is a good correspondence of estimates only when the effort in genetic sampling is high, which guarantees a high recapture probability. Juvenile wolves had higher annual CR survival

rates than adult wolves; kill rates most affected wolf survival in this natural ecosystem, and higher annual kill rates were correlated with higher annual adult survival. Adult survival (but not juvenile survival) was an important predictor of population growth rate. Hence, we show that kill rates indirectly affected population growth rate and directly affected adult survival rate. These reliable estimates of survival have unique value because the Isle Royale wolf population is not exposed to any human-caused mortality. Therefore, knowing long-term patterns of annual survival and its relationship to population growth rate for a not hunted wolf population represents a critical baseline for wolf conservation throughout its worldwide distribution.

**Keywords** Capture-recapture · Isle Royale · Microsatellite DNA · Non-invasive · Survival · Wolves

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## Introduction

Reliable estimates of abundance and survival rate are fundamentally important for understanding the dynamics of any animal population, particularly those of conservation concern. An increasingly important means of estimating abundance and survival is the use of non-invasively collected genetic samples in conjunction with the principles of capture–recapture theory (hereafter, genetic-CR estimates) (Schwartz et al. 2007).

One of the most important and persistent challenges associated with genetic-CR estimates is to understand their accuracy, especially in relationship to the accuracy of traditional field-based estimates. To this end, much effort has focused on theoretically evaluating the effects of genotyping errors on estimates of abundance (McKelvey and Schwartz 2004a, b; Waits and Leberg 2000). Another

important basis for understanding the accuracy of genetic-CR estimates is to make useful comparisons with traditional methods of estimation (e.g., Arrendal et al. 2007; Creel et al. 2003; Cubaynes et al. 2010; Guschanski et al. 2009; Kendall et al. 2009; Marucco et al. 2009; Solberg et al. 2006; Zhan et al. 2006). These comparisons indicated that genetic-CR based estimates of abundance tended to be 25–50 % greater than estimates based on traditional methods (Marucco et al. 2011); this measure can decrease with an increase in sampling effort (Stenglein et al. 2010).

The accuracy of genetic-CR estimates is also vulnerable to violations in standard assumptions associated with CR modeling, like individual capture heterogeneity or “trap-shy” and “trap-happy” behavioural responses. While these assumptions are well known in standard CR analysis, detection and evaluation of the causes of such violations in genetic-CR applications is more difficult than is commonly appreciated (Lukacs and Burnham 2005). In this context, a persistent obstacle to comparing genetic-CR estimates with traditional estimates is the inability to simultaneously estimate abundance with genetic-CR estimators and with traditional estimators that are believed to be reasonably accurate. In most cases, the reliability of the traditional-based estimates is unknown or dubious.

Here, we make such a comparison, involving estimates of abundance during each of 10 years for a small population of wolves (*Canis lupus*) living on an island where dispersal into and out of the population is negligible (Adams et al. 2011). Reliability of the field-based estimates is facilitated by unusual biogeographical and logistical circumstances. Both the island size (544 km<sup>2</sup>) and mean population size (~24 wolves) are small. The combination of small island size, small population size, and intensive period of observation represent unusually good conditions for accurately estimating abundance.

There is also international conservation value in providing reliable estimates of survival for this wolf population. Throughout the world, the greatest threat to wolf conservation is a high rate of human-caused mortality (Ciucci et al. 2007; Creel and Rotella 2010). The Isle Royale wolf population is not exposed to any human-caused mortality, therefore, knowing its long-term pattern of annual survival in relation to population growth rate provides a critical baseline for wolf conservation. Nevertheless, such baseline data is extremely rare (Creel and Rotella 2010), and particularly valuable within the Isle Royale ecosystem, because the “apparent CR survival ( $\Phi$ ) estimate”, which is usually estimated in CR studies of open populations where mortality and dispersal are confounded, can be considered “true CR survival” due to negligible emigration or immigration.

We estimated population size and survival of the Isle Royale wolf population over 10 years using both a

traditional aerial-based survey and a genetic-CR approach to: (1) evaluate the efficacy of new popular genetic-CR estimates and compare them to high effort traditional aerial-based estimates, which is the most commonly employed technique to census wolves in North America, (2) estimate true CR survival for an unexploited wolf population, (3) test what could affect survival, given the hypothesis that either kill rates, moose availability, or wolf density could be the major factors that could affect wolf survival in this natural ecosystem.

## Methods

### The study system

Isle Royale National Park (Michigan, USA) is a wilderness island (544 km<sup>2</sup>) in the northwest corner of Lake Superior where wolves (*Canis lupus*) have been studied annually since 1959. A centerpiece of this research has been observations made from aircraft during an intensive 7-week field season conducted each January and February. These surveys indicate that the wolf population is typically comprised of between 18 and 27 wolves (upper and lower quartiles), and that the population is usually organized into 3 or 4 packs and several loners (Peterson et al. 1998; Vucetich et al. 2002). Immigration and emigration are limited to periods in the winter when ice forms between the 20 and 30 km passage of water that separates Isle Royale from Canada. During the study period, an ice bridge formed only once in late February of 2008, after we had collected most of the scat samples for that winter season. Moreover, analysis of microsatellite DNA indicated that, during the study period (1999–2009), no immigration events occurred (Adams et al. 2011). Additionally, the Isle Royale wolf population is not exposed to any human-caused mortality.

### Field-based estimates of abundance and survival

The aerial observations involved a light, fixed-wing aircraft flown every day that weather permitted during the intensive 7-week field season conducted each January and February. In most years of the study period,  $\geq 1$  individual in each pack was collared. Between 1999 and 2009, we placed radio collars on a total of 20 wolves. Aided by radio telemetry, most packs were observed a dozen or more times each field season. Aerial observations also included high resolution digital photographs that aided in distinguishing individual wolves based on differences in pelage and relative size. From these methods of observation, we estimated the number of wolves in each pack. Lone wolves (i.e., those not belonging to any pack) were detected by their tracks in the snow. On sunny days, when tracks are

conspicuous, we systematically looked for and followed the tracks of any lone wolf until the wolf was observed directly. The identity of lone wolves was also aided by digital photography. We estimated annual abundance as the sum of pack sizes plus the number of lone wolves in the population. Because pack-living wolves sometimes spend time apart from the pack, observing a lone wolf required not only seeing a wolf on its own, but also observing the packs, at about the same time (i.e., on the same day or within several hours), with all of the wolves they were believed to include. Estimates of abundance, based on this method, have been published elsewhere for Isle Royale wolves (e.g., Vucetich et al. 2002). The method is liable to be particularly accurate when applied to Isle Royale because the population size is small and observed frequently during a long field season.

Annual survival rate, at the population level, was estimated from aerial observations. Specifically, we estimated the number of juveniles (9 months old) in each pack each winter on the basis of physical appearance and behavior, which were the new recruits into the population. We estimated the number of deaths ( $D_t$ ) to occur each year in the population as abundance in year  $t - 1$  ( $N_{field,t-1}$ ) minus  $N_{field,t}$  plus the number of juveniles observed in year  $t$ . Annual survival rate ( $S_{field,t}$ ) was estimated as  $1 - (D_t/N_{field,t})$ . The accuracy of this method depends greatly on being able to accurately distinguish juveniles from adults. Even for experienced observers, making this distinction from an aircraft can be difficult. Estimates of survival for Isle Royale wolves, based on this method, are described in Peterson et al. (1998).

Long-term research on Isle Royale also includes annual estimates of moose abundance, moose-to-wolf ratio, and kill rate (kg of prey per wolf per day) as described in Vucetich and Peterson (2004). We used these covariates in genetic-CR models aimed at explaining variation in rates of survival (see “Analytical Methods” Section).

#### Collection of genetic samples

The genetic-CR estimates are based on the analysis of wolf scats collected during the same winter field season when abundance and survival were estimated with field-based methods. Most of the analyzed scats (>95 %) were collected from kill sites, where wolves fed for several days and defecated frequently. Kill sites were detected from fixed-wing aircraft by direct observation and by following tracks in the snow (Peterson 1977). Kill sites were visited as soon as possible after the wolves abandoned the carcass.

Our sampling strategy involved collecting on average 7 ( $\pm 3$ ) times more scat samples than there are estimated number of wolves per pack. The field considerations that favour collecting this number of scats were: visiting as

many kill sites as possible, as soon as possible, and searching for as long as possible, given logistical constraints. When time is unlimited, we typically searched for scats until 10 min had passed without finding another scat. Weather conditions occasionally prevented us from visiting a kill site before snowfall made it impossible to find scats. The other scats were collected opportunistically (e.g., while snowshoeing through the forest, or by landing the plane after observing scat along a track that had been deposited on a frozen lake). Scats of lone wolves were collected along their tracks, or at kill sites, because lone wolves regularly scavenged from sites where pack had killed moose and left.

Because scats were deposited and collected during winter, they froze shortly after defecation. After being deposited, they remained frozen for between 24 h and 2 weeks, until a sample from a scat was collected, at which point it was stored in 95 % ethanol or DET buffer (Frantzen et al. 1998; Murphy et al. 2002). Preserved scat samples were stored at  $-20^\circ\text{C}$  for 12–96 months, until we extracted the DNA.

#### Laboratory methods

##### *DNA extraction, amplification and visualization*

DNA was extracted from fecal samples using the Qiagen Stool Kit protocol (Valencia, CA) in a laboratory dedicated to low quality and quantity DNA samples. One extraction negative was included with each DNA extraction to monitor for contamination. Probability of identity and the probability that siblings share the same genotype were calculated for 8 microsatellite loci (Waits et al. 2001) (ESM Table 1). The polymerase chain reaction (PCR) was used to generate multilocus genotypes at 8 microsatellite loci designed for canids (Holmes et al. 1995; Mellersh et al. 1997; Neff et al. 1999; Ostrander et al. 1993; Ostrander et al. 1995). The Qiagen Multiplex PCR Kit was used for multiplexing loci. PCR products were visualized on an Applied BioSystems 3730xl DNA Analyzer. Sex identification was determined by amplification at the microsatellite locus MS34A (Sundqvist et al. 2001). A fecal DNA extract was considered to have useable DNA template if amplification occurred at four or more loci during an initial PCR using all loci, and then subject to a second PCR using all loci. Those that amplified at three or fewer loci were removed from further analysis. Additional details are given in Online Resource 1.

##### *Data filtering*

Fecal DNA genotypes were screened for accuracy using a modified multiple tubes approach (Taberlet et al. 1997). Heterozygous results were accepted after each allele was

observed at least twice. Homozygous results were accepted after positive homozygous results were obtained three times (Adams and Waits 2007; Frantz et al. 2003). Samples for which a consensus genotype was obtained at seven or eight loci were included in further analyses. Sex was determined by three positive amplifications (male) or negative amplifications (female) at locus MS34A. Program Gimlet (Valiere 2002) was used to determine the number of unique individuals present within the consensus genotypes. If a genotype differed from another by one allele and the difference could be due to allelic dropout these genotypes were considered a tentative match until further PCR replication clarified the results. Additional details are given in Online Resource 1.

## Analytical methods

### *CR modeling*

Each 7-week winter field season represented a capture session. The study included 11 capture sessions (1999–2009). Each wolf scat, collected and genotyped successfully, was considered a “capture or recapture” of the individual genotyped on the day of collection. We constructed a capture history for each wolf by recording whether it was captured or not in each capture session. We analyzed these capture histories using program MARK (White and Burnham 1999) and UCARE 2.2 (Choquet et al. 2009).

We used a multi-session open population Cormack–Jolly–Seber (CJS) model to estimate apparent survival ( $\Phi$ ) and recapture rates ( $p$ ). The apparent CR survival rates, estimated from the CJS models, were considered true CR survival due to the negligible emigration or immigration of this isolated wolf population.

Following recommendations by Lebreton et al. (1992), we checked the goodness-of-fit of a fully parameterized model using UCARE 2.2. The overall test is composed of tests for transience (Pradel et al. 1997) and trap-dependence (trap-happiness or trap shyness; Pradel 1993), which are particularly sensitive to heterogeneity in recaptures and independence of individuals. Therefore, we fully investigated models only if robust to assumptions. We then defined a set of candidate models incorporating biologically relevant combinations of several temporal and individual effects on both survival and detection probabilities to avoid data dredging (Burnham and Anderson 2002).

We defined a set of a priori biologically plausible models, and we evaluated our models using a hierarchical approach where we first focused on models most likely to explain recaptures; and second, we held the best explanatory variables for recaptures constant while focusing on our primary goal: to determine factors influencing wolf survival.

Therefore, we wanted to first test the hypothesis that wolves’ characteristics and sampling effort could affect recapture rates. Adult wolves, which usually have a strong marking behavior, might be more frequently recaptured by scat collection along trails than young wolves (Marucco et al. 2009); however, our sampling design where the collection of scats was conducted at kill sites should have avoided this age effect. Hence, our hypothesis was that age, as well as sex, should not be an important predictor of recapture rates. The major hypothesis was that recapture rates could be affected by effort. We tested this hypothesis and examined the level and types of effort that could mostly affect recapture rates. We measured effort in different ways. First, we measured effort directly from the number of scats analyzed (called “scats analyzed”), and from the number of scats that gave successful genotypes (called “successful scats”). We also evaluated effort considering the average laboratory success rate per number of wolves; our rationale for measuring effort in this manner is: if an investigator knows even roughly how many animals are in the population ( $N$ ) and what the average lab success rate is, then this measure of effort (i.e. scat analyzed  $\times$  mean success rate/ $N$ ) will inform the investigator as to how many scats should be collected (called “scats per wolf”). We also tested whether the recapture probability was affected by kill rates, because the majority of the scats were collected at kill sites, therefore a higher kill rate could represent a higher opportunity to collect scats. These different measures of effort were included as time-dependent external covariates in a logit-linear relationship to recapture probability.

Then, our interest was to evaluate how annual estimates of survival were affected by wolf’s age, year, wolf density, kill rate, and moose:wolf ratio. Our hypothesis was that young wolves could have lower survival than adult, as detected by other studies (e.g. Fuller 1989; Hayes and Harestad 2000; Marucco et al. 2009). We also hypothesized wolf density would have a negative effect on survival due to a possible increase in intraspecific strife, which has been reported as one of the major cause of mortality in natural settings (Fuller et al. 2003); and that survival would be greater when moose:wolf ratio was greater and kill rates were higher, as suggested by Vucetich and Peterson (2004), which explained how the population growth rate of wolves was significantly influenced by moose density. Wolf density, kill rate, and moose:wolf ratio were considered time-dependent external covariates in a logit-linear relationship to survival probability. We categorized each wolf’s age as juvenile (9–10 months old), adult ( $\geq 21$  months old), or unknown. Each wolf was categorized into an age class using information derived from a combination of techniques (i.e. pedigree analysis, field data, collaring data, and direct observations of defecation).

Models were ranked and weighted according to the Akaike information criterion for small sample sizes (AICc, Burnham and Anderson 2002). We model averaged to obtain averaged parameter estimates and standard errors. We evaluated the importance of each covariate in explaining the parameter of interest and we measured this importance by the sum of Akaike weights over the subset of models that included that variable (Burnham and Anderson 2002). We estimated standard errors of the annual survival rates using the Delta Method (Seber 1982).

*Population size estimation*

We used recapture rates from the best CJS model to derive estimates of abundance using a Horvitz–Thompson-type estimator (McDonald and Amstrup 2001):

$$\hat{N}_t = \sum_{s=1}^n \frac{I_{st}}{\hat{p}_{st}} \tag{1}$$

where  $\hat{N}_t$  is the estimated abundance at time  $t$ ,  $I_{st}$  is 1 if animal  $s$  was captured during time  $t$  and 0 if it was not, and  $\hat{p}_{st}$  is the maximum likelihood estimate of the recapture rate of animal  $s$  at time  $t$ . The approximate variance of  $\hat{N}_t$  is:

$$\text{var}(\hat{N}_t) = \sum_{s=1}^n \left( \frac{I_{st}(1 - \hat{p}_{st})}{\hat{p}_{st}^2} + \frac{I_{st}\sigma_{p_{st}}^2}{\hat{p}_{st}^3} + \frac{I_{st}(1 - \hat{p}_{st})\sigma_{p_{st}}^2}{\hat{p}_{st}^4} \right) \tag{2}$$

This estimator corresponds to the canonical estimator, which is a count statistic divided by an estimate of detection probability (Williams et al. 2002).

We then compared three sets of abundance estimators, that is, estimates based on the number of unique genotypes detected each year, the CR-genetic based estimate (Eqs. 1 and 2), and the field-based estimates. We also compared two sets of survival estimators, those based on the best CJS model and the field-based estimate.

**Results**

*Aerial-based estimates of abundance and survival*

Estimates of population size, derived from aerial-based estimates, indicated an averaged population size of 24 wolves ( $\pm 5$ ) from 1999 to 2009. Population sizes varied from a minimum of 17 in 2002 to a maximum of 30 wolves in 2005 and 2006 (Table 1). Average number of juveniles was 7 ( $\pm 3$ ) from 1999 to 2009, and varied from a minimum of 4 in 2007 and 2008 to a maximum of 12 juveniles in 1999 and 2004. The averaged field-based estimate of survival rate was 0.71 ( $\pm 0.16$ ) from 1999 to 2009, and varied

from a minimum of 0.49 in 2001 to a maximum of 0.96 in 2000 (Table 1).

*Genetic-CR estimates of abundance, and survival*

Between 1999 and 2009, we visited 207 kill sites (74 % of those detected), and collected and analyzed 1,739 scats. We collected a mean of 8 scats per kill site. On average 158  $\pm$  84 scats were analyzed per year, with a minimum of 52 scats in 2000 and a maximum of 299 in 2005. Of the 1,739 scats analyzed, 826 scats gave successful results for an averaged 48.9 % ( $\pm 10.9$ ) success rate over the years. A total of 107 genotypes were detected, 42 females and 65 males. A negligible level of genotyping errors is indicated by 92.5 % of the genotypes being recaptured more than once (ESM Fig. 1). When we pooled the recaptures in each session for CR analysis, 60 % of the genotypes were recaptured more than once over the sampling sessions (ESM Fig. 1).

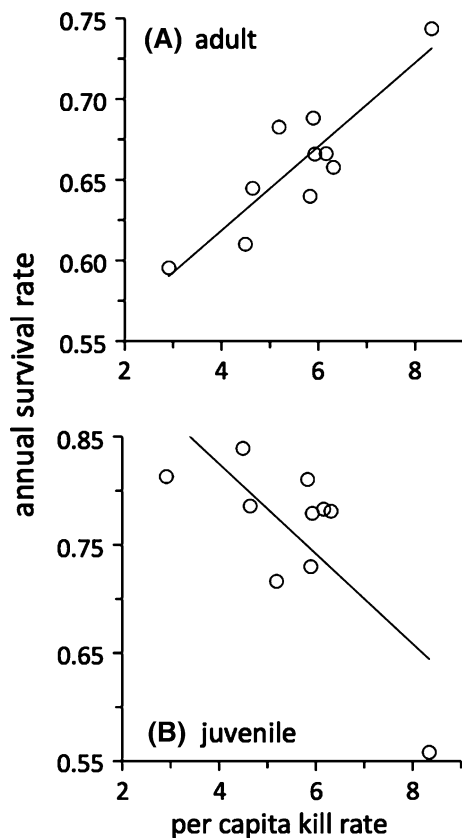
The overall goodness of fit test was not significant ( $\chi^2 = 19.948$ ,  $P = 0.524$ ) and showed no signs of transience ( $z = -1.325$ , two-sided test,  $P = 0.907$ ) nor of trap-dependence ( $z = -0.746$ , two-sided test,  $P = 0.456$ ), suggesting that the fully parameterized CJS model was appropriate. In particular, heterogeneity among individuals in detection probability was negligible.

The CJS best models were associated with several ecologically important results. The first two top models, with delta AICc < 2, included age, kill rate and an age-kill rate interaction term as the most important covariates affecting survival ( $\Phi$ ) and a measure of effort (scats per wolf) as the main factor affecting recapture rate ( $p$ ); the ratio of moose:wolves also explained an important part of variation for survival, while wolf density was less important (Table 2). In particular, the sum of the Akaike weights for kill rate was 0.31, and for moose:wolves was 0.26. In all models, age was best explained if adults and unknowns were pooled (suggesting that the unknowns were likely older than 1 year). The sum of the Akaike weights for age was almost 1.00, which indicated the importance of this variable in explaining the probability of survival. Juvenile wolves had higher annual survival rates than adult wolves for every year except 2002 (Table 1). Higher annual kill rate was correlated with higher annual adult survival (Fig. 1a). In particular, age ( $\beta = 8.62$ , SE = 5.28) and kill rates ( $\beta = 0.31$ , SE = 0.17) had positive effects on survival, while their interaction had a negative effect ( $\beta = -1.28$ , SE = 0.75), which indicated that at high kill rates, while adult survival increased, juvenile survival slightly decreased (Fig. 1b). In fact, juvenile and adult survival were negatively correlated ( $r = -0.91$ ,  $P < 0.001$ ) (Fig. 2). Adult survival was a predictor of wolf population growth rate ( $r = 0.63$ ,  $P = 0.046$ ) (Fig. 3), while juvenile survival was not ( $P = 0.26$ ).

**Table 1** Summary data for estimates of abundance and survival for Isle Royale wolves, 1999–2009

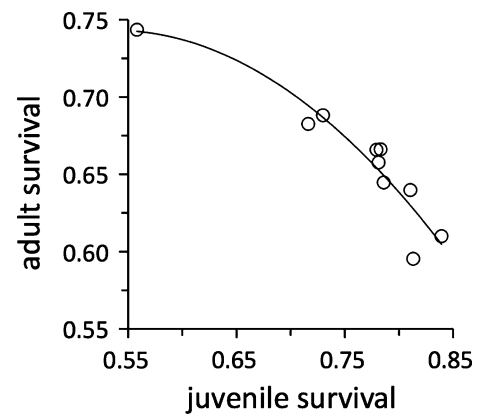
Years	$N_{\text{genetic-CR}}$	$N_{\text{genetic}}$	$N_{\text{field}}$	Juvenile $S_{\text{genetic-CR}}$	Adult $S_{\text{genetic-CR}}$	Average $S_{\text{genetic-CR}}$	Average $S_{\text{field}}$	Effort	Kill rate	Moose to wolf ratio
1999		25	25	0.79 (0.12)	0.65 (0.06)	0.71 (0.05)	0.93	2.09	4.64	39.9
2000	19 (6)	13	29	0.81 (0.12)	0.60 (0.12)	0.70 (0.05)	0.96	0.88	2.91	35.6
2001	27 (6)	14	19	0.72 (0.17)	0.68 (0.08)	0.70 (0.07)	0.49	1.83	5.19	59.0
2002	28 (7)	17	17	0.56 (0.21)	0.74 (0.09)	0.65 (0.08)	0.53	3.31	8.34	64.7
2003	17 (6)	13	19	0.73 (0.12)	0.69 (0.06)	0.71 (0.06)	0.71	2.75	5.90	47.4
2004	36 (9)	26	29	0.78 (0.08)	0.66 (0.06)	0.72 (0.05)	0.89	4.57	6.31	25.9
2005	39 (10)	35	30	0.81 (0.10)	0.64 (0.06)	0.72 (0.06)	0.66	4.87	5.83	18.0
2006	33 (9)	30	30	0.84 (0.11)	0.61 (0.07)	0.72 (0.05)	0.77	3.47	4.49	15.0
2007	32 (9)	27	21	0.78 (0.10)	0.67 (0.06)	0.72 (0.05)	0.57	5.58	6.16	18.3
2008	25 (8)	23	23	0.79 (0.09)	0.67 (0.04)	0.72 (0.05)	0.9	3.34	5.93	28.3
2009	31 (8)	25	24					2.20		22.1

$N$  is abundance, and  $S$  is survival. The subscripts “genetic-CR” refers to the final CJS model-averaged estimates and their corresponding standard errors; the subscript “genetic” refers to the number of unique genotypes detected each year, and the subscript “field” refers to estimates based on aerial surveys. Effort is (scat analyzed  $\times$  mean success rate/ $N$ ), and kill rate is kg of prey/wolf/day. Numbers in parentheses are standard errors

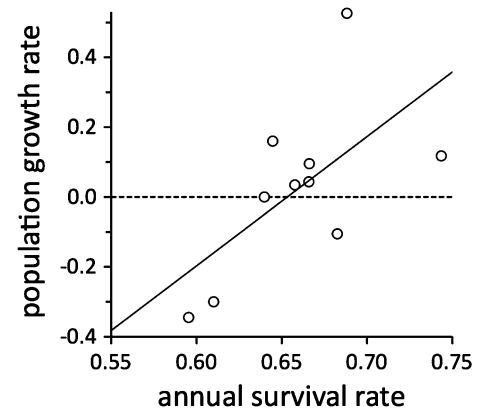


**Fig. 1** The relationship between adult (a) and juvenile (b) annual survival (based on the genetic capture–recapture method) and annual kill rate. Data are for the time period 1999–2009

Recapture rates were best explained by effort, in particular by “scats per wolf” which was calculated by (scat analyzed  $\times$  success rate/ $N$ ). The sum of the Akaike weights for this covariate is 0.56, indicating the high importance of this variable in explaining the probability of



**Fig. 2** The relationship between adult and juvenile annual survival (based on the genetic/capture–recapture method). Data are for the time period 1999–2009



**Fig. 3** The relationship between adult annual survival (based on the genetic/capture–recapture method) and annual population growth rate. Data are for the time period 1999–2009

**Table 2** Cormack–Jolly Seber models run in Program MARK to calculate maximum likelihood estimates of survival rates ( $\Phi$ ) and recapture rates ( $p$ ) of wolves in Isle Royale, 1999–2009

Model	AICc	Delta AICc	AICc weights	Likelihood	N
$\Phi$ (age $\times$ killrate) $p$ (scats per wolf)	398,387	0.000	0.215	1	6
$\Phi$ (age $\times$ moose:wolf) $p$ (scats per wolf)	398,989	0.601	0.159	0.7403	6
$\Phi$ (age $\times$ wolf density) $p$ (scats per wolf)	400,398	2.011	0.079	0.3659	6
$\Phi$ (age) $p$ (scats per wolf)	400,402	2.014	0.078	0.3652	4
$\Phi$ (age $\times$ moose:wolf) $p$ (scats analyzed)	400,653	2.265	0.069	0.3221	6
$\Phi$ (age) $p$ (scats analyzed)	401,053	2.665	0.057	0.2638	4
$\Phi$ (age $\times$ killrate) $p$ (scats analyzed)	401,168	2.780	0.051	0.249	6
$\Phi$ (.) $p$ (scats analyzed)	401,280	2.893	0.051	0.2354	3
$\Phi$ (age $\times$ moose:wolf) $p$ (successful scats)	402,195	3.807	0.032	0.149	6

Models are ranked according to their AICc values. Only models with delta AICc < 4 are shown

recapture (Table 2). Averaged estimates of recapture rates varied from  $0.52 \pm 0.13$  to  $0.92 \pm 0.05$  depending on the number of scats analyzed per year and on the averaged success rate. Effort (i.e. scats per wolf) was negatively correlated with CI width of adult CR survival estimates ( $r = -0.62, P = 0.04$ ), and there was not a significant correlation neither with CI width of juvenile CR survival estimates ( $r = -0.41, P = 0.24$ ), nor with CI width of CR abundance ( $r = -0.31, P = 0.39$ ).

Estimates of population size, derived from the best CJS models recapture rates, indicated an averaged population size of 29 wolves ( $\pm 7$ ) from 1999 to 2009 (Table 1). The genetic CR estimates indicated an averaged number of 10 juveniles ( $\pm 5$ ), and of 18 adults ( $\pm 6$ ) from 1999 to 2009 in Isle Royale.

#### Comparison of aerial-based and genetic-CR estimates

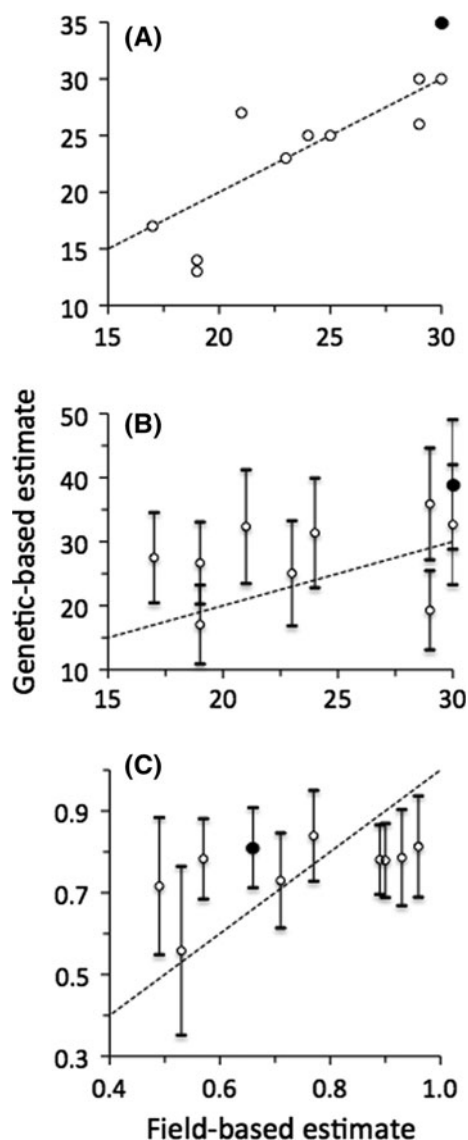
For the 10 year observation period, field-based estimates of abundance were well correlated with number of genotypes detected each year ( $r = 0.867, P = 0.001$ ; Table 1; Fig. 4a). Field-based estimates of abundance were not correlated with CR-based estimates ( $r = 0.454, P = 0.188$ ; Fig. 4b). Field based estimates were within one standard error of the CR-based estimates of abundance for 6 of the 10 years during our study period (Fig. 5). The year 2000 was associated with a limited number of scat samples ( $n = 52$ ) and the lowest recapture rate ( $0.52 \pm 0.13$ ). When estimates for the year 2000 were omitted, the correlation between field-based estimates and CR-based estimates of abundance was significant ( $r = 0.742, P = 0.022$ ). Field-based estimates of number of juveniles were well correlated with CR-based estimates ( $r = 0.848, P = 0.001$ ); while the number of adults were not correlated ( $r = 0.295, P = 0.204$ ). When estimates for the year 2000 were omitted, the correlation between field-based estimates and CR-based estimates of adult abundance was significant ( $r = 0.699, P = 0.018$ ).

Estimates of minimum abundance, derived from field estimates, indicated an averaged abundance of 24 wolves ( $\pm 5$ ), which is within the exhaustive estimates derived from the CR models ( $28 \pm 7$ ).

Survival estimated from field observations was uncorrelated with averaged survival estimated from CR-based methods ( $r = 0.420, P = 0.227$ ) (Fig. 4c). When estimates for the year 2000 were omitted, the correlation between field-based estimates and CR-based estimates of survival was still not significant ( $r = 0.511, P = 0.160$ ). Nevertheless, field-based methods and CR-based methods did not differ with respect to expected annual survival, averaged across years (i.e.,  $S_{\text{field}} = 0.71 \pm 0.16$  [SE];  $S_{\text{genetic-CR}} = 0.71 \pm 0.02$ ).

#### Discussion

The overall process of using non-invasive genetic techniques to estimate abundance and survival has rarely been tested and comparisons with traditional estimates in controlled settings have rarely been performed. Our genetic CR open population analysis to estimate wolf abundance and survival in Isle Royale over 10 years, while simultaneously estimating those parameters with traditional aerial-based techniques, gave important insights. We detected a good correspondence of genetic CR estimates and field estimates, and obtained low CI for genetic CR population parameters estimates only when effort in genetic sampling was high, which guaranteed a high recapture probability. Our measure of effort (i.e. scat analyzed  $\times$  mean success rate/ $N$ ), if  $>3$ , guaranteed good levels of precision for both abundance and survival estimates. This measure of effort may inform the investigator as to how many scats (or samples) should be collected for a desired level of precision, if the investigator knows even roughly how many animals are in the population ( $N$ ) and what the average lab



**Fig. 4** Comparison of field-based and genetic-based observations for number of individuals detected (**a**), number of individuals estimated from capture–recapture models (**b**), and estimated annual survival (**c**) for Isle Royale wolves, 1999–2009. More precisely, the *x*-axis in **a** and **b** is the number of wolves estimated in the field, the *x*-axis in **c** is the field-based estimate of survival. The *y*-axis in **a** is the number of genotypes detected, in **b** is the genetic/capture–recapture based estimate of abundance, and in **c** is the genetic capture–recapture based estimate of survival. The *dotted line* depicts the 1:1 line of equality. The *dark circles* represent estimates of year 2000

success rate is. High recapture rates are also important to minimize bias, such as presence of individual capture heterogeneity, and increased precision (Lebreton et al. 1992). Recapture rates varied from  $0.52 \pm 0.13$  to  $0.92 \pm 0.05$  depending on effort, indicating good levels of recapture for CR analysis.

The CR abundance estimates were, as expected, higher than and highly correlated to the number of genotypes detected, because the CR estimates are derived from the

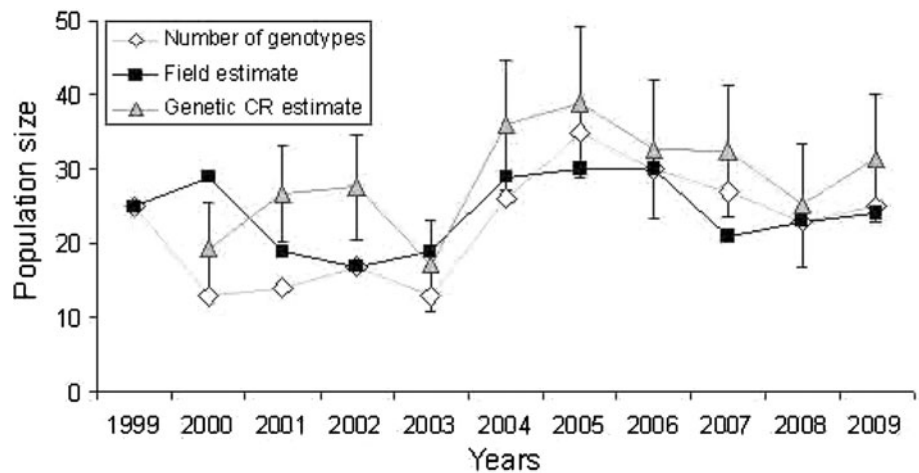
recaptured genotypes. Field estimates instead are independent minimum counts of wolves on the Island. CR estimates of annual abundance were higher than the field estimates for eight of ten years. This is expected, because CR estimates take undetected individuals into account. In wolves, packs are the primary social units of a wolf population, but the population is composed of lone wolves, typically dispersers, too (Fuller et al. 2003). Genetic CR modeling is more likely to take into account these individuals than is field counts. In fact, lone wolves are counted in the genotype estimate and are characterized by low recapture rates, which indirectly increases the overall CR population size estimate.

Higher field estimates have been detected only during 2000 and 2003, likely due to an adult genetic sampling underestimate. The number of adult genotypes detected in 2000 was only 30 % of the total observed in the field. This is more likely when a small number of scats is collected. In fact in this study, recapture rates were best explained by effort, which indicates that the accuracy of the CR estimates, strictly dependent on the recaptures, can be further improved by increasing the number of scats collected and successfully analyzed. Year 2000 is characterized by the lowest number of scats collected, as well as by the lowest number of adult wolves detected, and poor agreement on estimates. Therefore, a greater sample size not only increases precision, but also limits bias in underestimating the abundance. An increasing agreement between estimates was evident after 2003, indicating an improvement in the CR sampling over the years.

Absence of individual heterogeneity in recaptures indicated an adequate scat sampling strategy, where individual wolves were indifferently sampled, and scats had very low levels of genotyping errors. In fact, the recapture rates did not decrease with increased population size, and the estimates of population size did not increase with increased sample size. These two indices are good indirect checks indicating that there are no major genotyping errors in the dataset. Paetkau (2003) and Lukacs and Burnham (2005) reported that data sets which have not been heavily scrutinized both by geneticists and ecologists often show either geographical closure violations or presence of heterogeneity in recaptures without biological explanations (Lukacs and Burnham 2005). This study indicates that collecting scats at kill sites could minimize individual heterogeneity in recaptures (i.e. no signs of transience were detected in the fully parameterized CJS model), reducing also differences in recaptures between juveniles and adults (i.e. age was not an important explanatory variable for recapture rates). This genetic CR sampling strategy is therefore adequate for wolves or other carnivores that spend time and defecate at kill sites, which permit unbiased sampling of individuals in a pack. However, understanding the detection rate of lone wolves, for any



**Fig. 5** Estimated wolf population size in Isle Royale from winter 1999 to winter 2009. Population size was estimated with a genetic capture–recapture analysis ( $\pm$ SE) (CR estimate), the maximum number of genotypes detected, and a field estimate



method of estimating abundance is notoriously difficult. Nevertheless, much research suggests that lone wolves typically represent 10–15 % of a wolf population (Fuller et al. 2003). Our field-based and genetic-based estimates from Isle Royale are consistent with that composition, suggesting that lone wolves could be also sampled at kill sites while they are scavenging from sites where pack had killed moose and left, or occasionally along tracks.

CR estimates of survival were not well correlated with field estimates. CJS survival CR estimates are generally robust (Lebreton et al. 1992), and in particular here, as indicated by GOF tests and the good CIs. On the other hand, survival field estimates are likely to be less reliable, because they depend on the accurately distinguishing 9 month-old pups from adults, and that distinction is prone to error. This lack of correlation confirms, as had been suspected, that the field-based method of estimating survival was unreliable. A better correlation was detected if we compared juveniles CR survival and field observations. This is likely because juveniles are easier to recognize and often stay in the pack the following year allowing good field estimates of survival, while adult survival is more difficult to evaluate in the field. The exceptional case of a small and isolated population with almost no immigration or emigration allowed the evaluation of “true” CR survival estimates. In a study case, where the population of study is connected to others, one should refer to “apparent” estimates of CR survival, and not “true” survival, which is usually the case in CR studies of populations that are not isolated, where mortality and dispersal are confounded. Hence, an apparent CR survival rate will be lower than the true CR survival rate, because it is confounded by the dispersal rate.

True CR survival of juvenile wolves on Isle Royale was higher than adult wolves. We expected the opposite, with juvenile and inexperienced individuals having lower survival than adults, as detected by other North American studies (e.g. Fuller 1989; Hayes and Harestad 2000). In

human dominated environments, such as the European Alps (Marucco et al. 2009), and Italy (Lovari et al. 2007), juvenile wolves showed the lowest survival, likely due to the high level of poaching events and road-kills that usually affect the non-expert individuals. However, wolves on Isle Royale, where natural mortality is often due to intraspecific strife and starvation (Peterson et al. 1998), showed the opposite pattern, likely because juveniles tend to remain in their natal packs and are not targets during inter-pack conflicts (Mech and Boitani 2003). Few studies detected this pattern (e.g. Ballard et al. 1987, Gogan et al. 2000). With a larger dataset, it will be interesting to test the hypothesis that adult subordinates are the age class with lower survival on Isle Royale.

Kill rate and moose:wolf ratio best explained the trend in wolf survival over the years. Higher annual kill rates, and higher annual moose:wolf ratio, were correlated with higher annual adult survival. However, at high kill rates and moose:wolf ratio, while adult survival increased, juvenile survival slightly decreased. These interactions between age and kill rate, and age and moose:wolf ratio, may indicate that at the highest kill rates and moose:wolf ratio, overall adult survival may increase, leading to greater intra-pack competition for food. Therefore, this study suggests that pups remaining in their natal pack are buffered against starvation and are not subject to being killed in interpack conflicts. However, when the number of wolves increase in the pack, starvation and intraspecific strife may involve juveniles too. We also detected an important negative correlation between juvenile and adult survival in Isle Royale wolves, not entirely expected. The relationship is important, in part, because properly parameterized age-structured models require estimate of the correlations among age-specific vital rates, and one would tend to expect these correlations to be positive (Morris and Doak 2002). However, our results depict a strong negative relationship. We do not understand the mechanism underlying this relationship. However, it likely

involves how parental investment in different-age offspring varies with food availability and how mortality associated with adult dispersal also varies with food abundance.

Finally, adult survival (but not juvenile survival) was a predictor of population growth rate. The relationship between survival and population growth rate is one of the most fundamental relationships in demography (Sibly and Hone 2002). For some species, like ungulates, fluctuations in adult survival have a strong influence on population growth rate (Gaillard et al. 1998). Considerable evidence indicates that survival is an important predictor of growth rate for wolves in general (Fuller et al. 2003) and Isle Royale wolves in particular (e.g., Peterson et al. 1998). In this context it is important to note that survival appeared to have an important influence, even though it is not the sole determinant of growth rate and is only one of several potentially influential vital rates. The observed fit may indicate the extent to which recruitment can effectively compensate for survival.

### Recommendations and conservation implications

Estimating abundance and survival using non-invasively collected genetic samples in conjunction with the principles of capture-recapture theory is becoming an important tool for wildlife managers (Schwartz et al. 2007). Validating this approach is fundamental, especially if genetic CR estimates will be used for conservation and management purposes. The small, well-monitored population of wolves living on Isle Royale, provided the opportunity to compare genetic CR estimates with standard field estimates and to explore the specific genetic CR sources of error, largely discussed by other authors (Lukacs and Burnham 2005; Marucco et al. 2011). The presence of capture heterogeneity is one of the most difficult problems facing estimation of animal abundance using CR analysis (Pledger and Efford 1998). Problems of capture heterogeneity with wolves can arise during scat sampling due to differing behaviour of individuals leading to disparate probabilities of finding their scats. Our sampling strategy based on scats collected at kill site proved adequate to avoid this problem. With this design, we produced a dataset which met the assumption of homogeneity in recapture rates which is fundamental for having unbiased CR estimates, important for the conservation of small populations. However, a small sample size in year 2000 gave an inaccurate genetic CR abundance estimate, which indicates that the number of successful scats analyzed per wolf need to be adequate to guarantee a high recapture probability. Solberg et al. (2006) recommended that studies using non-invasive genetic methods based on faecal samples should aim at collecting 2.5–3 times the number of faecal samples as the “assumed” number of animals. Our measure of effort (i.e.

scat analyzed  $\times$  mean success rate/ $N$ ) proved useful and significant in measuring this and can be used in other studies. This genetic CR approach to estimate population size and survival can be widely applied to broad spatial and temporal scales for other elusive and wide-ranging species. It is interesting to notice that most studies which applied genetic CR techniques produced population size estimates 30–50 % larger than estimates obtained with traditional methods (e.g. Cubaynes et al. 2010; Guschanski et al. 2009; Kendall et al. 2009; Marucco et al. 2009; Solberg et al. 2006; Zhan et al. 2006). This is likely due to the application of CR analysis which accounts for undetected individuals, providing more accurate estimates. However, we discussed how inappropriate sampling designs and the presence of residual genotyping errors in final datasets used for CR analysis can cause overestimation of abundance. It is important in any study to carefully evaluate these aspects, especially if genetic CR estimates are used for management and conservation decisions.

Our assessment of the relationship between kill rate and mortality rate is important because one of the key assumptions of carnivore conservation is that the success of a conserved carnivore population is largely dependent on their access to food. Moreover, it is important to understand the long-term dynamics of such a relationship for a population that is not harvested by humans. The Isle Royale population is one of the few long-term studies of an unexploited wolf population and this survival analysis represent a critical baseline for wolf conservation throughout its worldwide distribution.

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### References

- Adams JR, Waits LP (2007) An efficient method for screening faecal DNA genotypes and detecting new individuals and hybrids in the red wolf (*Canis rufus*) experimental population area. *Conserv Genet* 8:123–131
- Adams JR, Vucetich LM, Hedrick PW, Peterson RO, Vucetich JA (2011) Genomic sweep and potential genetic rescue during limiting environmental conditions in an isolated wolf population. *Proc R Soc B* 278:3336–3344. doi:10.1098/rspb.2011.0261
- Arrendal J, Vila C, Björklund M (2007) Reliability of noninvasive genetic census of otters compared to field censuses. *Conserv Genet* 8:1097–1107
- Ballard WB, Whitman JS, Gardner CL (1987) Ecology of an exploited wolf population in south-central Alaska. *Wildl Monogr* 98:1–54
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York 488 pp

- Choquet R, Lebreton JD, Gimenez O, Reboulet AM, Pradel R (2009) U-CARE: utilities for performing goodness of fit tests and manipulating C-Apture-REcapture data. *Ecography*. 32:1071–1074
- Ciucci P, Chapron G, Guberti V, Boitani L (2007) Estimation of mortality parameters from (biased) samples at death: are we getting the basics right in wildlife field studies? A response to Lovari et al. (2007). *J Zool* 273:125–127
- Creel S, Rotella JJ (2010) Meta-analysis of relationships between human off-take, total mortality and population dynamics of gray wolves (*Canis lupus*). *PLoS ONE* 5(9):e12918. doi:10.1371/journal.pone.0012918
- Creel S, Spong G, Sands JL, Rotella J, Zeigle J, Joe L, Murphy KM, Smith D (2003) Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. *Mol Ecol* 12:2003–2009
- Cubaynes S, Pradel R, Choquet R, Duchamp C, Gaillard J-M, Lebreton J-D, Marboutin E, Miquel C, Reboulet A-M, Poillot C, Taberlet P, Gimenez O (2010) Importance of accounting for detection heterogeneity when estimating abundance: the case of French wolves. *Conserv Biol* 24(2):621–626
- Frantz AC, Pope LC, Carpenter PJ (2003) Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. *Mol Ecol* 12:1649–1661
- Frantzen MAJ, Silk JB, Ferguson JWH, Wayne RK, Kohn MH (1998) Empirical evaluation of preservation methods for faecal DNA. *Mol Ecol* 7:1423–1428
- Fuller TK (1989) Population dynamics of wolves in North-Central Minnesota. *Wildl Monogr* 105:1–41
- Fuller TK, Mech LD, Cochrane JF (2003) Wolf population dynamics. In: Mech LD, Boitani L (eds) *Wolves. Behavior, ecology, and conservation*. The University of Chicago Press, Chicago, pp 161–191
- Gaillard JM, Festa-Bianchet M, Yoccoz NG (1998) Population dynamics of large herbivores: variable recruitment with constant adult survival. *Trends Ecol Evol* 13:58–63
- Gogan PJP, Olexa EM, Thomas N, Kuhlen D, Podruzny KM (2000) Ecological status of gray wolves in and adjacent to Voyageurs National Park, Minnesota. USGS, NRM Science Center, Bozeman
- Guschanski K, Vigilant L, McNeilage A, Gray M, Kagoda E, Robbins MM (2009) Counting elusive animals: comparing field and genetic census of the entire mountain gorilla population of Bwindi Impenetrable National Park, Uganda. *Biol Conserv* 142:290–300
- Hayes RD, Harestad AS (2000) Demography of a recovering wolf population in the Yukon. *Can J Zool* 78:60–66
- Holmes NG, Dickens HF, Parker HL, Binns MM, Mellersh CS, Sampson J (1995) Eighteen canine microsatellites. *Anim Genet* 26:132–133
- Kendall KC, Stetz JB, Boulanger J, MacLeod AC, Paetkau D, White GC (2009) Demography and genetic structure of a recovering grizzly bear population. *J Wildl Manag* 73(1):3–17
- Lebreton JD, Burnham KP, Clobert J, Anderson DR (1992) Modeling survival and testing biological hypotheses using marked animals—a unified approach with case-studies. *Ecol Monogr* 62(1):67–118
- Lovari S, Sforzi A, Scala C, Fico R (2007) Mortality parameters of the wolf in Italy: does the wolf keep himself from the door? *J Zool* 272:117–124
- Lukacs PM, Burnham KP (2005) Review of capture–recapture methods applicable to noninvasive genetic sampling. *Mol Ecol* 14:3909–3919
- Marucco F, Pletscher DH, Boitani L, Schwartz MK, Pilgrim KL, Lebreton J-D (2009) Wolf survival and population trend using non-invasive capture–recapture techniques in the Western Alps. *J Appl Ecol* 46:1003–1010
- Marucco F, Boitani L, Pletscher D, Schwartz MK (2011) Bridging the gaps between non-invasive genetic sampling and population parameter estimation. *Eur J Wildl Res* 57:1–13
- McDonald TL, Amstrup SC (2001) Estimation of population size using open capture–recapture models. *J Agric Biol Environ Stat* 6(2):206–220
- McKelvey KS, Schwartz MK (2004a) Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. *J Wildl Manag* 68(3):439–448
- McKelvey KS, Schwartz MK (2004b) Providing reliable and accurate genetic capture–mark–recapture estimates in a cost-effective way. *J Wildl Manag* 68(3):453–456
- Mech LD, Boitani L (2003) *Wolves: behavior, ecology and conservation*. University of Chicago Press, Chicago 472 pp
- Mellersh CS, Langston AA, Acland GM, Fleming MA, Ray K, Wiefand NA, Francisco LV, Gibbs M, Aguirre GD, Ostrander EA (1997) A linkage map of the canine genome. *Genomics* 46:326–336
- Morris WF, Doak DF (2002) *Quantitative conservation biology: theory and practice of population viability analysis*. Sinauer Associates, Sunderland
- Murphy MA, Waits LP, Kendall KC, Wasser SK, Higbee JA, Bogden R (2002) Long-term preservation methods for brown bear (*Ursus arctos*) faecal DNA samples. *Conserv Genet* 3:435–440
- Neff MW, Broman KW, Mellersh CS, Ray K, Acland GM, Aguirre GD, Ziegler JS, Ostrander EA, Rine J (1999) A second-generation genetic linkage map of the domestic dog, *Canis familiaris*. *Genetics* 151:803–820
- Ostrander EA, Sprague GF, Rine J (1993) Identification and characterization of dinucleotide repeat (ca)n markers for genetic-mapping in dog. *Genomics* 16:207–213
- Ostrander EA, Mapa FA, Yee M, Rine J (1995) One hundred and one new simple sequence repeat-based markers for the canine genome. *Mamm Genome* 6:192–195
- Paetkau D (2003) An empirical exploration of data quality in DNA-based population inventories. *Mol Ecol* 12:1375–1387
- Peterson RO (1977) *Wolf ecology and prey relationships on Isle Royale*. *Scient. Monogr. Ser. No. 11*, U.S. National Park Service, Washington, DC
- Peterson RO, Thomas NJ, Thurber JM, Vucetich JA, Waite TA (1998) Population limitation and the wolves of Isle Royale. *J Mammal* 79(3):487–491
- Pledger S, Efford M (1998) Correction of bias due to heterogeneous capture probability in capture–recapture studies of open populations. *Biometrics* 54:888–898
- Pradel R (1993) Flexibility in survival analysis from recapture data: handling trap-dependence. In: Lebreton J-D, North PM (eds) *Marked individuals in the study of bird population*. Birkhäuser Verlag, Basel, pp 29–37
- Pradel R, Hines JE, Lebreton J-D, Nichols JD (1997) Capture–recapture survival models taking account of transients. *Biometrics* 53:60–72
- Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. *Trends Ecol Evol* 22(1):25–33
- Seber GAF (1982) *The estimation of animal abundance and related parameters*, 2nd edn. MacMillan, New York
- Sibly RM, Hone J (2002) Population growth rate and its determinants: an overview. *Philos Trans R Soc Lond B* 357:1153–1170
- Solberg KH, Bellemain E, Drageset OM, Taberlet P, Swenson JE (2006) An evaluation of field and non-invasive genetic methods to estimate brown bear (*Ursos arctos*) population size. *Biol Conserv* 128:158–168
- Stenglein JL, Waits L, Ausband D, Zager P, Mack C (2010) Efficient, noninvasive genetic sampling for monitoring reintroduced wolves. *J Wildl Manag* 74:1050–1058

- Sundqvist AK, Ellegren H, Olivier M, Vila C (2001) Y chromosome haplotyping in Scandinavian wolves (*Canis lupus*) based on microsatellite markers. *Mol Ecol* 10:1959–1966
- Taberlet P, Camarra JJ, Griffin S, Uhres E, Hanotte O, Waits LP, DuboisPaganon C, Burke T, Bouvet J (1997) Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Mol Ecol* 6(9):869–876
- Valiere N (2002) Gimlet: a computer program for analysing genetic individual identification data. *Mol Ecol Notes* 2(3):377–379
- Vucetich JA, Peterson RO (2004) The influence of prey consumption and demographic stochasticity on population growth rate of Isle Royale wolves *Canis lupus*. *Oikos* 107:309–320
- Vucetich JA, Peterson RO, Schaefer CL (2002) The effect of prey and predator densities on wolf predation. *Ecology* 83(11):3003–3013
- Waits JL, Leberg PL (2000) Biases associated with population estimation using molecular tagging. *Anim Conserv* 3:191–199
- Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol Ecol* 10:249–256
- White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study* 46:120–138
- Williams BK, Nichols JD, Conroy MJ (2002) Analysis and management of animal populations. Academic Press, San Diego, 817 pp
- Zhan XJ, Li M, Zhang ZJ, Goossens B, Chen YP, Wang HJ, Bruford MW, Wei FW (2006) Molecular censusing doubles giant panda population estimate in a key nature reserve. *Curr Biol* 16(12):R451–R452