A novel assessment of population structure and gene flow in grey wolf populations of the Northern Rocky Mountains of the United States

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Abstract

The successful re-introduction of grey wolves to the western United States is an impressive accomplishment for conservation science. However, the degree to which subpopulations are genetically structured and connected, along with the preservation of genetic variation, is an important concern for the continued viability of the metapopulation. We analysed DNA samples from 555 Northern Rocky Mountain wolves from the three recovery areas (Greater Yellowstone Area, Montana, and Idaho), including all 66 re-introduced founders, for variation in 26 microsatellite loci over the initial 10-year recovery period (1995–2004). The population maintained high levels of variation (H_O = 0.64–0.72; allelic diversity k = 7.0–10.3) with low levels of inbreeding (F_{IS} < 0.03) and throughout this period, the population expanded rapidly (n_{1995} = 101; n_{2004} = 846). Individual-based Bayesian analyses revealed significant population genetic structure and identified three subpopulations coinciding with designated recovery areas. Population assignment and migrant detection were difficult because of the presence of related founders among different recovery areas and required a novel approach to determine genetically effective migration and admixture. However, by combining assignment tests, private alleles, sibship reconstruction, and field observations, we detected genetically effective dispersal among the three recovery areas. Successful conservation of Northern Rocky Mountain wolves will rely on management decisions that promote natural dispersal dynamics and minimize anthropogenic factors that reduce genetic connectivity.

Keywords: conservation genetics, grey wolf, migrant detection, migration, population assignment

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Introduction

Re-introduction is an important conservation tool for the recovery of threatened and endangered species (Griffith *et al.* 1989; Bangs & Fritts 1996). Recovery strat-

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egies that establish a network of large, genetically diverse founding populations (Wolf *et al.* 1996; Forbes & Boyd 1997; Miller *et al.* 1999; vonHoldt *et al.* 2008) and provide opportunities for individual exchange among them can help counteract the negative effects of genetic drift, small population size, and isolation common to newly formed populations (Allendorf 1983; Keller & Waller 2002; Vila *et al.* 2003; Riley *et al.* 2006).

The establishment and maintenance of genetically diverse and connected subpopulations are vital to long-term viability (Frankham 1996) and is an accepted conservation goal of recovery programmes (McNeely *et al.* 1990; USFWS 1994, 2008). Consequently, a critical evaluation of re-introduction success requires not only knowledge of biological and demographic parameters of the recovering populations, but an understanding of their genetic structure and connectivity (Wolf *et al.* 1998; Miller *et al.* 1999; Breitenmoser *et al.* 2001; Frankham *et al.* 2002).

Re-introduced populations often share a common founding pool of individuals and therefore, genetic methods that might otherwise be used to measure genetic exchange cannot confidently identify migrants and admixed individuals because they share a recent common ancestry. This problem is acute in two of the three recovery populations of grey wolves in the Northern Rocky Mountains (NRM; Fig. 1a). After a decade of protection under the Endangered Species Act (ESA 1973), wolves began naturally recolonizing the NRM through dispersal from Canada (Ream & Mattson 1982), resulting in a population of 80–100 individuals (8–10 breeding pairs) in the northwest Montana recovery area (Montana) by the mid-1990s (Fritts *et al.* 1995). Two recovery populations were established by re-introducing 66 Canadian-born wolves to Yellowstone National Park (Yellowstone) and central Idaho during 1995–1996, along with the translocation of 10 individuals from Montana to Yellowstone in 1997. Wolf re-introduction followed a federal plan to restore wolves to the Greater Yellowstone Area (GYA: Yellowstone, Southwest Montana, and Northwestern Wyoming) and Idaho recovery area (Idaho; USFWS 1994; Bangs & Fritts 1996; Fig. 1A). The re-introduction strategy divided wolves captured from wild Canadian packs equally between Idaho and Yellowstone for their release. Because wolf packs are comprised of closely related individuals (Lehman *et al.*

1992; Mech & Boitani 2003; vonHoldt *et al.* 2008), this re-introduction strategy resulted in the dispersion of genetically similar individuals into each of the two founding populations (Fig. 1b). Consequently, the ability to detect genetically effective migration between Idaho and GYA is confounded by shared close relatives among founding populations.

The three core recovery areas (GYA, Idaho, and Montana) are considered subpopulations of the NRM metapopulation (Fig. 1A; USFWS 2008; Oakleaf *et al.* 2006; USFWS *et al.* 2007) and following analysis and peer-reviewed comments, USFWS established a population recovery goal of a minimum of 30 breeding pairs and 300 individuals distributed among the three subpopulations for at least three consecutive years, and connected by genetically effective dispersal (i.e. migrants that successfully reproduce). Population recovery goals were reached in 2002 (USFWS 1994, pp. 6:75, 2008) contributing to delisting in 2008 (USFWS 2008). A critical requirement of delisting and metapopulation dynamics was genetically effective dispersal between recovery areas. Wolves are highly mobile and can disperse great distances (Ballard *et al.* 1983; Gese & Mech 1991; Boyd & Pletscher 1999; Mech & Boitani 2003), and the three NRM recovery areas are well within the known dispersal capabilities of wolves. In fact, routine wolf movement among the three recovery areas, as well as with adjacent Canadian populations, has been documented through radio telemetry monitoring of individuals, particularly between Idaho and Montana (USFWS 2008). However, the degree to which this movement translates into genetically effective dispersal was not well established. One evaluation of genetic structure within the NRM region found no evidence for natural gene flow into Yellowstone during the 10-year period following wolf re-introduction (vonHoldt *et al.* 2008).

Here, we developed a novel approach to identify migrants between the three recovery areas using micro-
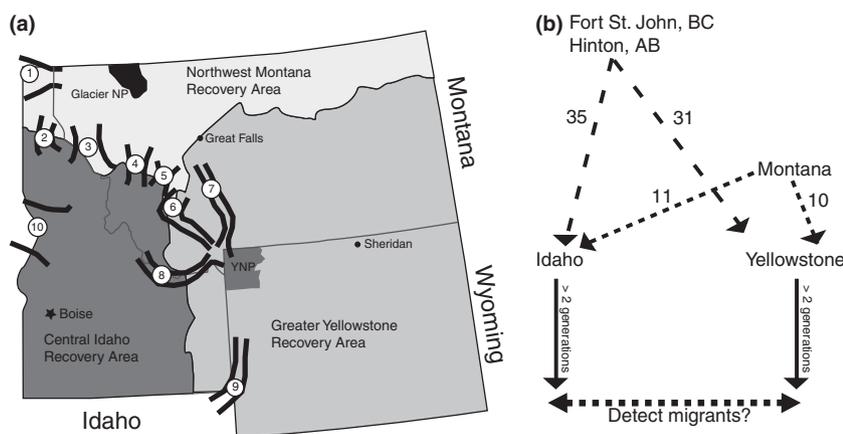

Fig. 1 (a) Northern Rocky Mountain grey wolf recovery areas and previously identified dispersal corridors (1–10; from Oakleaf *et al.* 2006; adapted from USFWS *et al.* 2007); (b) The re-introduction strategy of dividing founders between Idaho and Yellowstone (YNP; the numbers of individuals translocated are indicated).

satellite data. First, we assessed and compared overall levels of genetic variation at the founding (1995–1997), colonization (1998–2000), and contemporary (2001–2004) phases of a 10-year recovery period of wolf populations within the NRM. Second, we assessed population structure and differentiation using individual-based Bayesian analyses. Third, we used assignment tests, private alleles, and direct reconstruction of sibship to identify migrants and their admixed offspring. We used observation data to further verify the genetic assignments and determined a minimum number of migrants and genetically effective dispersal (e.g. admixture) events that have occurred over the first decade of recovery. These results were used to assess whether gene flow between subpopulations had occurred within the NRMs. Furthermore, we provided recommendations and methodology that may guide conservation and management of wolves in this region.

Materials and methods

Sample collection and molecular analysis

Because our objective was to reveal patterns of differentiation and migration between the three recovery areas, each area was used as a study population for all analyses. The Idaho and GYA recovery areas were founded in 1995 and 1996 by the release of 66 wolves relocated from Alberta and British Columbia, Canada. The GYA population was further augmented in 1997 with 10 individuals that were translocated from the Sawtooth pack of Montana (Bangs *et al.* 1998); a naturally recolonized population of wolves that dispersed south from Canada (Forbes & Boyd 1997). Consequently, GYA represents a mixture of wolves genetically related to wolves from the other 2 recovery areas.

Genetic samples were collected from 656 individual wolves between 1995 and 2004 from the two re-introduced populations and the naturally recolonized population. GYA and Idaho are represented by 30 wolf packs. All founding individuals of 1995 and 1996 were sampled prior to their release in Yellowstone and Idaho. Montana is represented by 19 wolf packs. During our study, the wolf population expanded rapidly with estimated annual census sizes ranging from 101 to 835 wolves throughout the entire study region (Idaho range = 14–452 wolves; GYA range = 21–324 wolves; and Montana range = 49–108; USFWS *et al.* 2005). The proportion of individuals genetically sampled throughout our study varied between recovery areas and was approximately 30% of total population size, ranging from approximately 75% during the early years (1995–1997), 50% during the middle years (1998–2000), and <21% in the later part of our study (2001–2004). All

samples were genotyped for a panel of 26 microsatellite loci (see Data S1) isolated from the domestic dog genome (see Data S1; J. Halverson in Neff *et al.* 1999; Breen *et al.* 2001; Guyon *et al.* 2003). Loci were selected to minimize physical linkage. The final data set used for analyses included individuals genotyped for at least 70% of the 26 loci typed (GYA, $n_{\text{Yellowstone}} = 262$ and $n_{\text{Wyoming}} = 73$; Idaho, $n = 116$; Montana, $n = 104$; Fig. 1).

Genetic variation

We defined three recovery phases for a temporal analysis of genetic variation; founding, colonization, and contemporary. The founding phase (1995–1997, $n_{\text{Idaho}} = 39$, $n_{\text{Montana}} = 45$, $n_{\text{GYA}} = 84$) included any known natural or management translocation events to Idaho and GYA, as the influx of potential breeders could influence levels of variation. The colonization phase (1998–2000, $n_{\text{Idaho}} = 87$, $n_{\text{Montana}} = 47$, $n_{\text{GYA}} = 103$) defined a period of population expansion and an increased likelihood for intra- and inter-population dispersal. The contemporary phase (2001–2004, $n_{\text{Idaho}} = 85$, $n_{\text{Montana}} = 104$, $n_{\text{GYA}} = 210$) defined a period after some evidence of localized population stabilization. In the majority of the GYA, population stabilization appeared to occur because of a paucity of available territories and high population density after 2001, and although Idaho and Montana experienced continued population expansion, we applied this temporal phase classification for all populations. Analysis of these three phases allows a more detailed analysis of the temporal dynamics of genetic variation in a recovering wolf population.

We used Cervus (Marshall *et al.* 1998) to calculate annual population-based heterozygosity, allelic diversity, polymorphic information content (PIC), and deviations from Hardy–Weinberg equilibrium for 26 microsatellite loci. The observed heterozygosity is estimated by dividing the total number of heterozygotes by the total number of individuals typed, and the multilocus expected heterozygosity is calculated and averaged across all loci using Nei's unbiased formula from allele frequencies assuming Hardy–Weinberg equilibrium (Nei & Tajima 1983; Marshall *et al.*1998). Deviations from Hardy–Weinberg equilibrium were assessed with chi-square tests and Bonferroni-corrected significance tests in Cervus. Linkage disequilibrium (LD) was determined for each recovery phase using GENEPOP (Raymond & Rousset 1995). Pairwise LD calculations were performed for 26 loci and specified MC parameters (1,000 dememorizations and 100 batches with 1000 iterations per batch) with Bonferroni correction (Rice 1989). Inbreeding coefficients (F_{IS}) were estimated using FSTAT v2.9.3.2, and Bonferroni-corrected significance levels (Rice 1989; Goudet 2001).

Population structure

To assess population structure and admixture, we used a Bayesian model-based clustering analysis with Structure v2.2 (Pritchard *et al.* 2000). We employed a mixed approach to determine the maximum-likelihood estimate of the number of genetic clusters (K) independent of locality information. A previously identified problem of using Structure on species with complex population structure is determining the true value of K (Pritchard *et al.* 2000; Pritchard & Wen 2004; Evanno *et al.* 2005). Specifically, the presence of closely related individuals in the sample data set will lead to inflated K-values, as suggested clusters under higher K-values represent known family groups and lineages. Therefore, we did not rely only on the optimal number of clusters K, but rather choose K based on the geographical concordance of individual's ancestry probabilities and the smallest K that captures structure in the data while showing small differences in likelihood values (Pritchard *et al.* 2000; Pritchard & Wen 2004). Using the general admixture model, parameter settings were as follows: 50 0000 burn-in and 500 000 Markov Chain Monte Carlo (MCMC) repetitions with 10 iterations. Structure was used to analyse all samples (1998–2004) and for each recovery phase to obtain the probability of ancestry (Pr) to each genetic cluster for each individual. Ancestries were considered when the probability surpassed an arbitrary threshold ($Pr > 0.80$). We employed CLUMPP v1.1.1 (Jakobsson & Rosenberg 2007) to amalgamate the Pr matrices of K iteration. We used the *Greedy* algorithm with 10 000 random permutations. CLUMPP accounts for inconsistencies in cluster labelling across random iterations and multimodality, or nonsymmetrical modes across permutations (Pritchard *et al.* 2000).

Migrant and admixture analysis

We used three genetic tests [prior assignment model in Structure (ST); sibship as determined from COLONY (CO; Wang 2004a); and private alleles (PA); see below] to classify individuals as either *migrant* (not originating from the sampled population), *admixed* (offspring of a migrant), or *nonmigrant* (originating from the sampled population). We analysed control samples with known histories (see next section) to evaluate each genetic test independently and jointly to determine concordance with known population origin. We further integrated observational data (e.g. date of birth/death, sampled as juveniles in a known subpopulation, radio-telemetry and known dispersal data) when available to confirm or negate genetic results. Observational data may also include putative parent–offspring relationships inferred from field-based behavioural data (vonHoldt *et al.*

2008). For field-based parentage assignments, we used Cervus v3.0 to confirm parentage (Marshall *et al.* 1998).

Admixture analysis. We used founding phase individuals as a reference population and assessed admixture up to two-generations using the Bayesian prior assignment model in Structure at $K = 3$ (Pritchard *et al.* 2000). Using population information and the ancestry probabilities of nonfounding individuals, we assigned each individual to the status of *nonmigrant*, *migrant*, or *admixed* when comparing the assignment probability and the population from which that sample originated. If an individual's assignment probability was high ($Pr > 0.8$), it was considered a confident population assignment, whereas inconclusive assignments ($0.5 < Pr < 0.8$) were regarded as consistent with *admixture*. If an individual's assignment was concordant with the sampled locality, it was assigned the *nonmigrant* status; conversely, assignments discordant with the sampled locality ($Pr > 0.8$ to a population other than sampled locality) were considered *migrants*. Burn-in and MCMC parameters are as described in the population structure analysis, with one iteration for $K = 3$.

Sibship analysis. Given the high degree of relatedness within wolf packs (vonHoldt *et al.* 2008), *nonmigrants* and *migrants* are expected to share sibship ties only to their natal populations. Thus, sibship ties of *nonmigrants* should be concordant with their sampled locality, whereas sibship ties of *migrants* should be discordant with their sampled locality. In contrast, *admixed* individuals should show sibship with two or more populations. We analysed sibship using two methods. First, COLONY v1.3 was used to create multigenerational nested sibship groups using a maximum-likelihood approach to estimate relationships from genotype data of groups of individuals (Wang 2004a). We used a standard frequency for null alleles and genotyping error rate (0.05; J. Wang, personal communication). We analysed all nonfounders to increase correct inference of full and half-sibling relationships. Second, to further support sibship groups for cases where COLONY is ambiguous, pairwise relatedness values were estimated across the entire data set using KINGROUP (Konovalov *et al.* 2004). We used a strict significance threshold ($P < 0.01$) to identify individuals having significant levels of relatedness.

Private allele analysis. A final genetic test used private alleles found only in founders of one of the three recovery populations. Individuals whose private alleles derived only from their sample locality were considered *nonmigrants*. Those individuals who had at least two

private alleles deriving only from a population different from their sampled population were considered *migrants. Admixed* individuals possessed at least two alleles private to a population different from their sampled population and at least one allele private to their sampled population. These criteria for private alleles may be conservative, but we followed this procedure to reduce false-positives attributed to potential genotyping error, homoplasy, or mutation (e.g. Marshall *et al.* 1998) and to more fully use the limited number of private alleles found in our analysis.

Private allele analysis was assessed using GENALEX (Peakall & Smouse 2006), which identified alleles private to each population during the founding phase (1995–1997). We included individuals from the Montana ($n = 45$) sampled through 1997, but restricted analysis of Idaho and Yellowstone to their founders ($n_{Idaho} = 35$; $n_{Yellowstone} = 31$). We chose to retain the population membership of the translocated Sawtooth individuals in Yellowstone as Montana, because we focused our analysis on natural occurring migration between the three recovery areas. We tabulated the presence of private alleles in the remaining nonfounder samples.

Evaluating detection methods with empirical date

We identified 103 known *nonmigrants*, 11 known *migrants* and 34 known *admixed* individuals as controls. The *nonmigrant* individuals were either sampled as a juvenile (<10 months of age and unlikely to disperse) or had been previously genetically verified as offspring of known nonmigrants (vonHoldt *et al.* 2008). We genetically confirmed Idaho parentage of two *nonmigrant* Idaho wolves (B108 and B111) and two *nonmigrant* Wyoming individuals that were sampled as juveniles (FA2P59 and FA3G11). Ninety-nine *nonmigrant* individuals were sampled in Yellowstone and had genetically verified parentage. Of the 11 known *migrants*, we included nine individuals that were translocated from Montana to GYA (Sawtooth pups: 064F, 065F, 066M, 067F, 068F, 069M, 070M, 071F, 072M) and a known disperser from Montana into Idaho (90-13) and from Idaho into Montana (F31208). We also included 30 first-generation *admixed* offspring (F1; $n_{Idaho} = 11$, $n_{GYA} = 19$) and 4 second-generation *admixed* offspring (F2) from GYA confirmed by genetic parentage analysis.

All control samples were assessed with three genetic tests (ST, CO, and PA), and results were reported independently and concordance noted when possible (e.g. where an individual had sufficient private alleles and relatives were detected). When an individual had conclusive results on more than one test, we report those cases where results were concordant.

Results

Temporal patterns in genetic variation

All loci were polymorphic in each recovery phase with low null allele estimates (<0.05) and showed high levels of allelic diversity ($k = 7.0$–10.3), which generally increased over the 10-year study period (Table 1; Supporting Tables S1–S3). The founding populations had high levels of allelic diversity (Idaho: $n = 33$, $k = 8.6$, $H_O = 0.700$; Yellowstone: $n = 31$, $k = 8.3$, $H_O = 0.705$; Table 2). As population expansion occurred during the colonization phase ($n_{Founding} = 168$; $n_{Colonization} = 237$; $n_{Contemporary} = 399$; Table 1), observed heterozygosity remained high (Idaho, $H_{Founding} = 0.708$, $H_{Colonization} = 0.717$, $H_{Contemporary} = 0.724$; Montana, $H_{Founding} = 0.651$, $H_{Colonization} = 0.636$, $H_{Contemporary} = 0.650$; GYA, $H_{Founding} = 0.706$, $H_{Colonization} = 0.715$, $H_{Contemporary} = 0.708$). Loci did not significantly deviate from Hardy–Weinberg equilibrium for all populations in any phase (Supporting Tables S1–S3). However, some loci deviated within subpopulations across the recovery phases (1995–1997, $n_{Idaho} = 1$, $n_{Montana} = 1$; 1998–2000, $n_{Idaho} = 3$, $n_{Montana} = 2$; 2001–2004, $n_{Idaho} = 2$, $n_{Montana} = 3$, $n_{GYA} = 3$), as expected given the presence of relatives in the sample. From a total of 325 pairwise comparisons, we identified pairs of loci that were significant for LD for each population in each recovery phase. After corrections for multiple tests, we found 118 pairs across all populations that were significant for the founding phase, 134 pairs for the colonization phase, and 171 pairs for the contemporary phase. Linkage disequilibrium is likely due to population structure, as the loci were not physically linked. We identified 12 alleles private to Idaho, 16 alleles private to Montana and 20 alleles private to Yellowstone during the

Table 1 Allelic diversity (k), sample size (N), polymorphic information content (PIC), observed and expected heterozygosity (H_O and H_E, respectively; 26 microsatellite loci)

Phase	N	k	H_O	H_E	PIC
Founding phase					
Idaho	40	8.5	0.708	0.760	0.719
Montana	45	7.4	0.651	0.711	0.665
GYA	84	9.7	0.713	0.763	0.730
Colonizing phase					
Idaho	87	9.4	0.717	0.766	0.731
Montana	47	7.0	0.636	0.670	0.653
GYA	103	9.4	0.714	0.741	0.706
Contemporary phase					
Idaho	85	9.5	0.723	0.767	0.733
Montana	104	9.1	0.650	0.728	0.690
GYA	210	10.3	0.708	0.738	0.705

Table 2 Allelic diversity (*k*), sample size (*N*), observed and expected heterozygosity (H_O and H_E, respectively), and polymorphic information content (PIC) for the founder populations of Yellowstone National Park and Central Idaho (26 microsatellite loci; *Significant deviation from HWE in the CID founders only, $P < 0.01$)

Locus	Yellowstone (*N* = 31)				Idaho (*N* = 34)			
	k	H_O	H_E	PIC	*k*	H_O	H_E	PIC
Pez5	5	0.613	0.655	0.587	4	0.853	0.669	0.597
Pez8	8	0.839	0.786	0.744	9	0.794	0.813	0.776
Pez12	9	0.839	0.814	0.775	8	0.706	0.793	0.751
Pez15	7	0.71	0.779	0.731	9	0.618	0.811	0.773
Pez19	5	0.29	0.359	0.324	3	0.412	0.442	0.353
FH2001	9	0.71	0.815	0.776	8	0.676	0.755	0.711
FH2004	13	0.871	0.874	0.845	12	0.735	0.849	0.817
FH2010	6	0.613	0.629	0.555	6	0.735	0.746	0.697
FH2054	8	0.613	0.83	0.795	10	0.824	0.834	0.798
FH2088	7	0.645	0.669	0.605	6	0.676	0.709	0.655
FH2137	8	0.774	0.778	0.729	8	0.794	0.802	0.761
FH2324	8	0.774	0.831	0.794	8	0.824	0.851	0.818
FH2611	11	0.774	0.894	0.868	12	0.941	0.883	0.857
FH2658	6	0.806	0.762	0.711	7	0.824	0.818	0.781
FH2670	10	0.935	0.864	0.834	11	0.706	0.876	0.849
FH2766	5	0.516	0.766	0.711	5	0.706	0.728	0.664
FH2785	8	0.806	0.738	0.688	7	0.676	0.717	0.672
FH2790	5	0.742	0.662	0.604	5	0.559	0.561	0.502
FH2869	5	0.258	0.319	0.301	5	0.176	0.402	0.373
FH2914	8	0.71	0.847	0.813	7	0.706	0.769	0.721
FH3047*	5	0.548	0.764	0.711	5	0.324	0.728	0.672
FH3398	11	0.839	0.883	0.855	12	0.794	0.867	0.839
FH3399	11	0.774	0.819	0.782	11	0.824	0.841	0.81
FH3725	14	0.839	0.883	0.856	10	0.735	0.767	0.731
FH3853	14	0.645	0.896	0.871	11	0.765	0.808	0.773
FH3965	18	0.839	0.923	0.901	18	0.824	0.927	0.907
Average	8.6	0.705	0.763	0.722	8.3	0.700	0.760	0.718

founding phase (Supporting Table S4). Inbreeding coefficients (F_{IS}) remained low for all recovery phases ranging from −0.04 for GYA during the colonization phase, to 0.02 for Montana in the contemporary phase.

Population structure

Determining the optimal number of population clusters (*K*) using Structure is not straightforward when complex population structure is present (Pritchard *et al.* 2000; Pritchard & Wen 2004; Evanno *et al.* 2005). Inflated likelihood values for higher *K*-values (e.g. *K* > 5) are likely attributed to the partitioning of related founders in different subpopulations, as well as an enrichment of relatives in each population (vonHoldt *et al.* 2008). We observed that the least amount of variation in Ln P(X|K) from 10 iterations was consistent for *K* = 3 (Supporting Fig. S1). Higher *K*-values suggested clustering concordant with known wolf pack lineages, particularly in the better-sampled Idaho and GYA populations (Fig 2). This result has been seen in other

population structure studies on wolves (e.g. Aspi *et al.* 2006), as well as other species with complex structure or data sets consisting of closely related individuals (e.g. Berry *et al.* 2004; Bergl & Vigilant 2007). Consequently, we choose lower *K*-values (*K* = 3 and 5) and amalgamated the *Pr* matrices to best display the genetic variation at a regional scale (Fig. 2). Furthermore, Structure results revealed discernable structure at levels consistent with known population history and relatedness, and that at higher levels of *K*, genetic lineages of family groups could be identified. Once we established this, our use of *K* = 3 with individuals assigned to their sampling locality (i.e. recovery area) allowed us to use Structure's migrant detection option to determine the probability that an individual's origin was its sampling locality.

During all the recovery phases, *K* = 2 corresponded with the grouping of the re-introduced populations compared to the naturally recolonized population of Montana. Known dispersers (wolf 90–13) and translocated wolves (Sawtooth pack) were resolved at *K* = 3
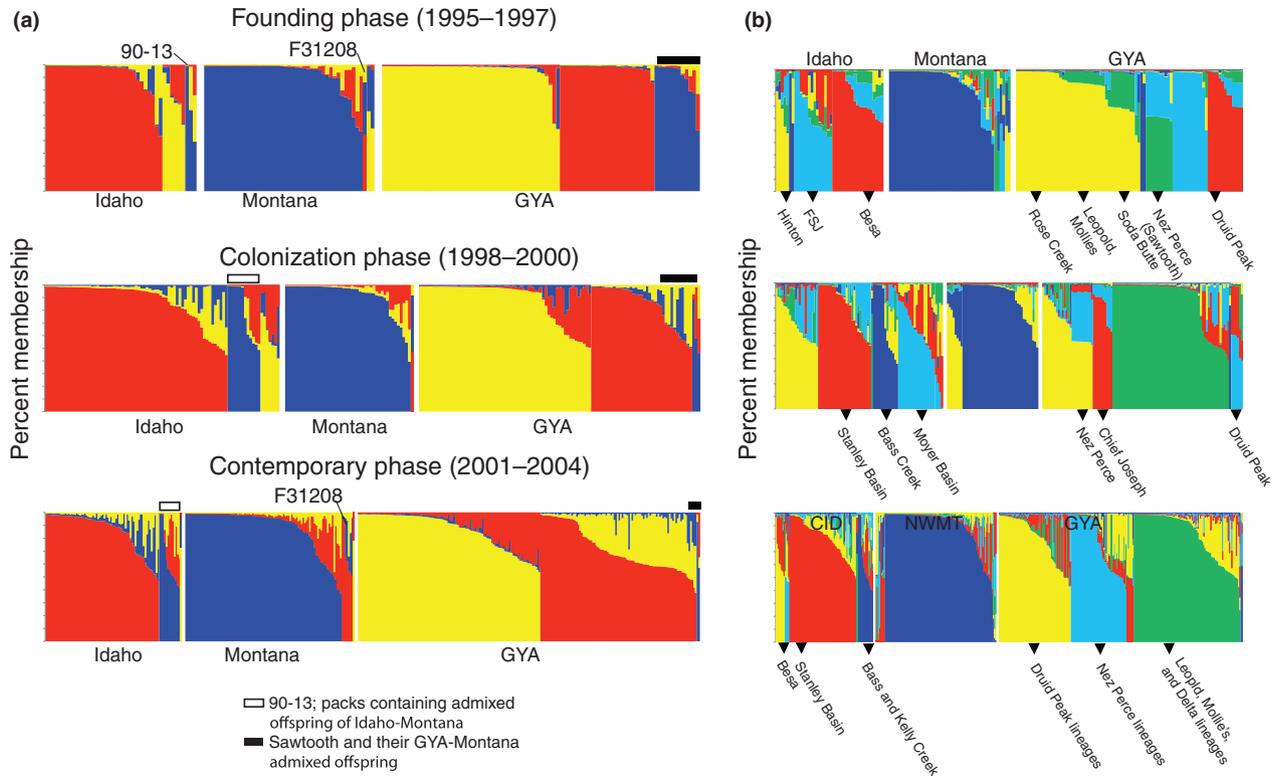
Fig. 2 Population structure analysis and posterior probability assignment to each genetic cluster for each individual from the three recovery areas. (a) Proportional membership to each genetic cluster assuming three genetic clusters ($K = 3$). Symbols indicate examples of positive controls in the migrant detection method for each recovery phase. (b) Proportional population memberships when five populations are assumed ($K = 5$). Here, pack-level memberships are resolved within populations and shared ancestry among the re-introduced populations over the three recovery phases. Hinton: Hinton, Alberta and FSJ: Fort St. John, BC.

(Fig. 2). The unique cluster assignments of Nez Perce individuals at $K = 5$ was likely due to the reproductive status of translocated 072M in the Nez Perce pack who originated from the Sawtooth pack in Montana and a founder female (048F) from the Fort St. John source region (vonHoldt *et al.* 2008; Fig. 2). Further, the population assignments of Nez Perce were shared with relatives from packs in Montana (Little Wolf, Boulder, and Graves Creek packs; $K = 3$, mean $Pr = 0.73$; $K = 4$, mean $Pr = 0.89$; $K = 5$, mean $Pr = 0.60$).

The known disperser from Montana to Idaho (wolf 90–13) and his offspring was clearly detected in Idaho having >97% assignment to Montana across all phases (Fig. 2). These offspring of wolf 90–13 also displayed a moderate assignment to Montana (average range $Pr = 0.40–54$ across K) as a signal of admixed ancestry. Another known migrant from Idaho to Montana was detected, although with an increased variation in assignment probabilities ($0.31 < Pr < 0.63$; Montana wolf F31208). In addition, the Bass Creek pack of Idaho shows high assignment to Montana ($Pr > 0.98$), a pack known to include first-generation *admixed* offspring of a disperser from Montana. Idaho's Thunder Mountain

pack also includes putatively *admixed* offspring from a pre-re-introduction Idaho wolf. Their average assignments are indicative of admixed ancestry across K-values (average range $Pr = 0.36–0.49$). Idaho's Kelly Creek and Thunder Mountain packs continued to display admixed ancestry with partial assignments to Montana (contemporary phase average $Pr = 0.38–0.48$).

Migrant and admixture detection: control samples

Using a single genetic test on control individuals with known *migrant* status, COLONY assigned the highest number of samples correctly ($n_{NM} = 102/103$, $n_{MIG} = 11/11$, $n_{AD} = 34/34$; Table 3). Private alleles assigned all individuals correctly, but the test could only be applied to fewer individuals ($n_{NM} = 63/63$; $n_{MIG} = 9/9$, $n_{AD} = 23/23$) because of the limited number of private alleles. Structure misassigned many *nonmigrants* (19/73) and *admixed* individuals (18/33) but otherwise could generally be applied to more individuals than private alleles (ST: $n_{NM} = 54/73$, $n_{MIG} = 7/7$, $n_{AD} = 15/33$). Structure misassigned 19 *nonmigrants* and 18 *admixed* GYA individuals to Idaho, suggesting that

Table 3 Assignment of controls ($n = 148$) based on three genetic tests (ST, Structure; CO, sibship; PA, private allele). For each genetic test the following is indicated from left to right: total number tested, results concordant with known sample history, and number verified by at least one other genetic test. The *migrant* status was assigned using Structure when $Pr > 0.8$, and the *admixed* status for partial assignments ($0.5 < Pr < 0.8$). NM, nonmigrant; MIG, migrant; AD, admixed

	NM ($n = 103$)	MIG ($n = 11$)*	AD ($n = 34$)
ST	73[†]:54:54	7:7:7	33[‡]:15:12
CO	103[§]:102:88	11:11:9	34:34:33
PA	63:63:63	9:9:9	23:23:23

*All assigned to their source population Montana from which they were translocated to GYA; [†]Nineteen GYA individuals misassigned to Idaho with high probability (ST; $Pr > 0.8$); [‡]Misassignments of 18 individuals between Idaho and GYA; [§]One GYA individual misassigned with Idaho.

the *nonmigrants* and *admixed* status is less well resolved between these populations with Structure than with COLONY or private alleles tests. A high proportion of assignments were also verified by an additional genetic test (ST > 80%, CO > 82%, PA = 100%; Table 3).

These findings suggest three important considerations concerning the application of these assignment tests. First, Structure has a high frequency of misassignments between Idaho and GYA, which probably reflects high relatedness among the founders used to establish both populations. Therefore, Structure may not detect actual admixture events between Idaho and GYA; rather, misassigments reflect shared ancestry (see discussion in Pritchard *et al.* 2000). Second, although COLONY performed well, it is frequency-based and if the proportion of population sampling is low, construction of nested sibling groups may be biased (Wang 2004a). As a result, admixed lineages may be missed if sufficient relatives are not included. Therefore, a negative COLONY result should not be evidence against admixture because our sample may not have included relatives. In contrast, a positive result should be interpreted as supporting admixture. Likewise, we have only a limited set of private alleles, and the genome of migrants or admixed individuals may not happen to sample these alleles.

These considerations suggest standards for individual classification. First, because we found higher levels of discordant results when using Structure, we rely more heavily on COLONY and private alleles when assessing migrant status. Second, we consider individual tests with COLONY and private alleles as most reliable when confirmed by an additional genetic test or observational data. In general, we regard COLONY as the most efficacious test because more individuals in our sample can be classified using this approach and assignments were accurately predicted in our sample of known individuals.

Migrant and admixture detection: noncontrol samples

We applied our multiple test detection method to assign all nonfounder samples ($n = 261$). We included observational data where available. Using COLONY as a single genetic test, we identified 243 *nonmigrants*, 2 *migrants*, and 13 *admixed* individuals (Table 4; Supporting Table S5). The two *migrants* comprised a Montana individual (wolf FA2H3) assigned to Idaho and one Idaho individual (wolf B99) assigned to GYA. FA2H3 was sampled in Glacier NP. Observational data have previously documented dispersal events from Idaho to Glacier National Park. Seven of 13 individuals had Idaho/Montana ancestry and four individuals had Idaho/GYA ancestry. Two Montana individuals (wolves A40425 and A40427) sampled prior to the re-introduction showed sibship with one Wyoming individual (wolf FA2P74) of the Greybull pack. These 2 assignments are likely an artefact that derives from Idaho wolves that existed prior to re-introduction as a result of dispersal from Montana, and subsequent direct dispersal or dispersal of their offspring to Wyoming. Private allele analysis identified three Idaho individuals (one assigned to Montana, two assigned to GYA) and one Montana individual assigned to GYA as migrants and 4 admixed individuals. Of the admixed individuals, there was one Idaho individual with Montana ancestry, one Montana individual with GYA ancestry, and two

Table 4 Assignment of wolves of unknown migrant status ($n = 261$) based on three genetic tests (ST, Structure; CO, sibship; PA, private allele). For each genetic text the following is indicated from left to right: the number assigned to each migrant category and number verified by at least one other genetic test. The *migrant* status was assigned using Structure when $Pr > 0.8$, and the *admixed* status for partial assignments ($0.5 < Pr < 0.8$). NM, nonmigrant; MIG, migrant; AD, admixed

	n	NM	MIG	AD
ST	253	151:147	34*:0	68[†]:10
CO	258	243:183	2[‡]:2	13:12
PA	110	100:98	4[§]:0	4:4

*Thirty-four GYA individuals were assigned to Idaho ($Pr > 0.8$); [†]The majority of admixture was found between CID and GYA ($n = 51$) and the rest were misassignments between Idaho and Montana ($n = 17$), some having observational support; [‡]One GYA individual assigned to Idaho and one Montana individual assigned to Idaho by sibship inferred from CO; [§]One Idaho with 2 GYA private alleles, two Idaho with 2 Montana private alleles, and one Montana with 2 GYA private alleles.
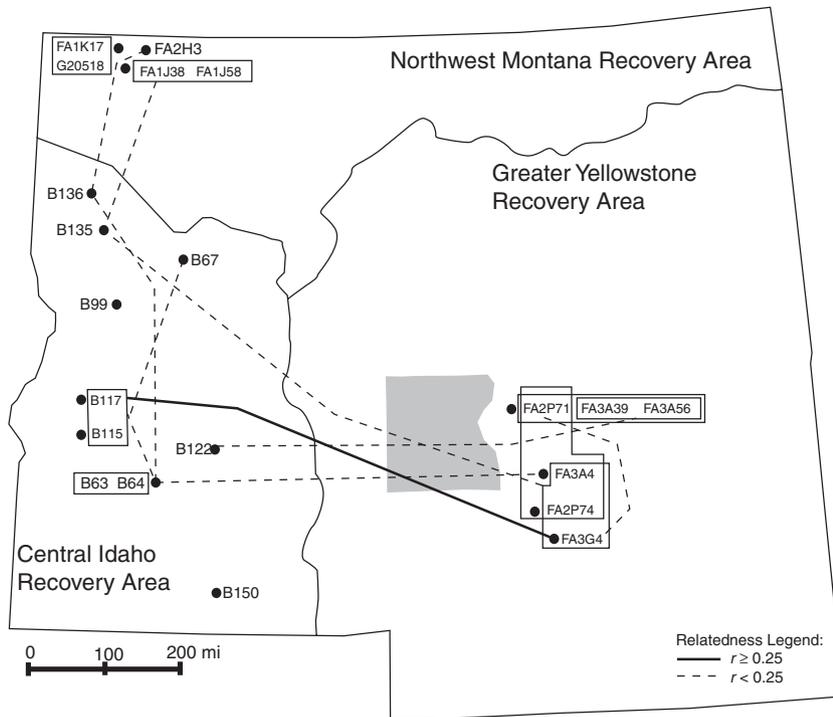
Fig. 3 Geographical location of putative migrants and admixed individuals (black circles indicate capture location and boxed samples indicate probably full- or half-sibling groups per recovery area, where $r > 0.25$ (see main text and Supporting Table S5 for details). Only significant sibship ties ($P < 0.05$) are indicated.

Idaho individuals with GYA ancestry. Finally, Structure found 34 *migrants* and 68 *admixed* individuals (85 were detected between Idaho and GYA), none confirmed by observational data. This high number probably reflects the sharing of close relatives between Idaho and GYA, rather than admixture.

Focusing on the concordance of multiple tests (COLONY, relatedness, structure, observations, and private alleles), we identified 23 individuals as *migrants* or *admixed* (Idaho: $n_{AD} = 6$, $n_{MIG} = 4$; Montana: $n_{AD} = 5$, $n_{MIG} = 2$; GYA: $n_{AD} = 6$; Supporting Table S5; Fig. 3). For example, Idaho wolf B99 was found to have GYA ancestry based on the COLONY genetic test and confirmed by Structure. B99 also shared significant pairwise relatedness with individuals from Glacier National Park of Montana. Montana wolf FA2H3 showed Idaho ancestry based on COLONY grouping with Idaho wolf B14. Wolf B14 was known to disperse from Idaho to Montana. In addition, FA2H3 was significantly related to F31208 ($r = 0.50$, $P < 0.0001$), a known disperser from Idaho's Indian Creek pack to Montana's Boulder pack.

Eight individuals showed signals of Idaho/Montana *admixed* ancestry (Supporting Table S5). Observational data on known dispersers between Idaho and Montana (e.g. 90–13 and B14) corroborate these results. Six GYA individuals showed signals of Idaho or Idaho/Montana ancestry, with four of these individuals being significantly related to Idaho wolf B58, a wolf of known Idaho/Montana ancestry that dispersed from Idaho to

the Greybull pack of Wyoming. Consequently, genetically effective dispersal involving reproduction is apparent in each population. Additionally, all individuals in this study could be assigned to either Montana origin or re-introduced GYA and Idaho wolves, providing no genetic evidence for immigration of individuals outside of the NRMs, or for a remnant ''native'' wolf population throughout the NRM that some have claimed were present prior to the 1980s Montana recolonization and 1995 and 1996 re-introductions (Urbigkit 2008).

Discussion

Genetic variation

The genetic health of the NRM wolf population is a critical issue in the conservation of grey wolves in the northwestern United States and has general implications for the recovery of large vertebrates. Because of broad regional sampling, intense monitoring, and knowledge of founding population histories, we were able to evaluate genetic diversity over the first decade of population recovery. We found that genetic diversity was high and maintained throughout the study period for the three recovery areas. Lower levels of genetic variation in Montana were probably attributable to its small population size relative to the other recovery areas. Additionally, despite the potential for a strong

founding bottleneck resulting from the re-introduction, we observed high levels of genetic variation in Idaho and GYA. This finding probably reflected a relatively large and genetically diverse founding population coupled with rapid population expansion. Overall, genetic diversity throughout the NRM was comparable or greater than estimates for other grey wolf populations (e.g. Roy *et al.* 1994; Jedrzejewski *et al.* 2005; Musiani *et al.* 2007) and was similar to estimates for other parts of the study area (Forbes & Boyd 1996; vonHoldt *et al.* 2008).

Inbreeding coefficients were low throughout the study period ($F_{IS} < 0.03$), which indicated a lack of significant inbreeding in each population. In addition to a rapid population expansion and a genetically diverse founding population, low inbreeding estimates were probably driven and maintained through behaviourally mediated inbreeding avoidance (see vonHoldt *et al.* 2008). Moreover, the presence of reproductively successful migrants between recovery areas may have influenced genetic diversity. Given recent patterns of genetic variation and demography for each recovery area, our results imply that genetic variation was maintained in the NRM wolf populations during our study.

Population structure of NRM wolves

We evaluated population structure across a large regional landscape. Using the Bayesian approach of Structure, we identified significant genetic structure for NRM wolves (Fig. 2). Much of the structure at $K > 3$ reflected genealogical lineages resulting from high reproductive output of specific wolf packs early in the recovery process especially in the GYA. For example, partitioning at $K = 5$ in GYA largely corresponded to specific wolf packs (e.g. Besa and Druid Peak packs; Rose Creek and Crystal Creek packs; Fig. 2B). Similarly, translocated individuals from Montana's Sawtooth pack to Yellowstone and Idaho were identified in the founding and colonization phases (Fig. 2). Offspring of some of these individuals were identified as admixed in Structure representing breeding with resident wolves. Specifically, two of the translocated Sawtooth wolves became breeders in Yellowstone's Nez Perce pack, which resulted in a unique genetic profile that can be clearly identified in subsequent phases in the GYA (Fig. 2b).

We found that wolves in the NRM do not represent a panmictic population, and instead corroborated previous findings of genetic subdivision among wolf populations on a regional scale (Roy *et al.* 1994; Musiani *et al.* 2007; Carmichael *et al.* 2008; Aspi *et al.* 2009). If populations experienced substantial gene flow, then divergence and genetic partitioning were expected to

decrease (Hartl & Clark 1997; Pritchard *et al.* 2000). Despite close proximity of regional subpopulation cores (~200 km apart) within established dispersal capabilities of wolves (Mech 1987; Gese & Mech 1991; Mech & Boitani 2003), population divergence appeared to have increased towards the end of the study period (Fig. 2).

In addition to the biological and environmental effects on population structure common to North American wolf populations (Roy *et al.* 1994; Geffen *et al.* 2004; Musiani *et al.* 2007; Carmichael *et al.* 2008; Muñoz-Fuentes *et al.* 2009), genetic differentiation in NRM wolves may be influenced by anthropogenic factors and studies done over the first decade of re-introduction have documented similar effects (Oakleaf *et al.* 2006; Murray *et al.* 2010; Smith *et al.* 2010). For example, while high-quality core habitat exists for wolves throughout much of the NRM study area, high human and livestock densities, as well as greater human access, characterize the areas surrounding and connecting each recovery area (Oakleaf *et al.* 2006). In an analysis of the habitat linkage and colonization probabilities between the three recovery areas, Oakleaf *et al.* (2006) found that Idaho and Montana have higher connectivity than either of these areas has to the GYA. This finding was corroborated by dispersal patterns of radio-collared wolves as greater dispersal occurred between Idaho and Montana than between either of these areas and GYA (Oakleaf *et al.* 2006). Further, regional-scale patterns of survival and mortality (Murray *et al.* 2010; Smith *et al.* 2010) for NRM wolves during the first decade of recovery showed increased mortality risk and lower survival for yearlings, dispersers, and wolves living in areas of overlap with private land and livestock. These demographic and spatial dynamics, which are largely driven by anthropogenic factors, may be critical to metapopulation dynamics of NRM wolves as they influence the rates of natural dispersal and genetic connectivity between recovery areas. Applying a landscape genetic approach that integrates spatially explicit genetic data with information on natural (e.g. topography, habitat type) and anthropogenic landscape features (e.g. livestock, private land, road density) is one method that could be used to evaluate the factors influencing gene flow in this region (Manel *et al.* 2003).

NRM wolf populations: migration and gene flow

Dispersal dynamics and the associated demographic parameters of survival, mortality, and habitat connectivity are integral to metapopulation dynamics in NRM wolves (Oakleaf *et al.* 2006; Murray *et al.* 2010; Smith *et al.* 2010). Field study of dispersal distances and dispersal rates in NRM wolves suggest high potential for adequate genetic connectivity through natural

processes alone (Forbes & Boyd 1997; Boyd & Pletscher 1999; USFWS unpublished data), but the relationship between these demographic characteristics and genetic differentiation following a decade of wolf recovery in the NRM was unknown. Importantly, dispersal and genetically effective migration are two different entities; the former generally being higher than the latter if migrants are incapable of reproducing because of social strife, lack of breeding positions, or decreased survival.

Quantifying levels of genetically effective migration between populations is a constant challenge in conservation genetics (Varvio *et al.* 1986; Slatkin 1987), particularly for populations not in equilibrium or having shared colonization histories (Slatkin 1993; Forbes & Boyd 1997). Because of the possibility of false-positives from a single test, we integrated results across multiple tests (assignment tests, sibship patterns, relatedness, private alleles and field observations). This approach was highly successful as known *nonmigrants, migrants,* and *admixed* individuals were identified in our control sample set. Moreover, a surprising finding was that sibship patterns classified correctly more of the unknown sample than widely used assignment tests. This result suggests that analysis of sibship can be an effective tool to assess genetically effective migration among closely related populations.

Our analyses detected *migrants and admixed* individuals in the three recovery areas and demonstrated genetically effective dispersal (Supporting Table S5). Specifically, we detected 21 individuals of putative *migrant* or *admixed* status over the course of the 10-year study, disregarding two Montana individuals sampled prior to the re-introduction (Supporting Table S5). Considering the significant levels of relatedness among these individuals, we identified probable family groups of siblings and assessed their sibship ties (Fig. 3; Supporting Table S5). Idaho wolves B99 and B150 lacked significant sibship ties. Consequently, all these individuals minimally defined eight unique family groups and assuming 4.16 years per generation (spanning 2.4 generations in this study; vonHoldt *et al.* 2008), 3.3 effective migrants per generation was the minimum number of genetically effective migration events that would explain our data. When we include our known migrants or admixed offspring from positive controls, our estimate increased to a minimum of 5.4 effective migrants per generation for our study period. Further, all the six GYA *admixed* individuals detected were sampled in Wyoming outside Yellowstone (Supporting Table S5), four of which were significantly related to Idaho dispersing wolf B58 who settled southeast of Yellowstone. This finding is informative with respect to patterns of dispersal and source-sink dynamics (Pulliam 1988), which have been suggested for our study area based on

a recent survival analysis (Smith *et al.* 2010). Wolves are almost completely protected in Yellowstone and their population dynamics are different than other parts of the study area (USFWS *et al.* 2010). High wolf densities and territory saturation in Yellowstone during the height of this study probably limited the ability of individuals to effectively disperse into this core area (vonHoldt *et al.* 2008). Consequently, our detection of admixture in GYA indicates that effective dispersal was most successful outside of Yellowstone during our study, presumably owing to greater opportunities to establish territories and breed. However, since 2004, we have observed migrants copulating with Yellowstone wolves; these dispersal events coincide with decreasing Yellowstone wolf densities (Yellowstone Wolf Project, NPS, unpublished data).

Our results should be viewed as a conservative minimum of the true number of migrants per generation in the NRM. Contrary to past studies on carnivores (e.g. Forbes & Boyd 1997; Cegelski *et al.* 2003) that used indirect estimates of gene flow (e.g. Slatkin's private allele method, Slatkin 1985; Wright's estimate N_m, Wright 1943), our approach focused on recent rather than historic levels of gene flow. However, because of the incomplete sampling of all three recovery populations and likely dispersers, we can only estimate a minimum number of migrants needed to explain the number of admixed individuals. The actual value is dependent on the fraction of all three populations sampled and the degree of relatedness among population members. Our data set represents approximately 30% of total censused NRM population over the course of the study period, based on annual year-end population estimates. Given this sampling fraction, the true number of migrants and levels of gene flow is presumably greater than our estimates for conditions through 2004.

Conservation and management implications

The role of genetics in endangered species recovery, science, and management has rarely been more prominent than for grey wolf recovery in the American West. Delisting of the NRM grey wolf required regional genetic connectivity to be demonstrated as well as specific population targets to be reached, and acceptable management plans from Idaho, Montana, and Wyoming (USFWS 2008). With recovery goals achieved in 2002, and approved state plans completed in 2007, wolf delisting first occurred in 2008 (USFWS 2009). However, due in part to the undetermined status of regional genetic connectivity, delisting was remanded back to the USFWS in 2008 for further consideration. This legal judgment highlighted the need for a critical evaluation of metapopulation dynamics with respect to genetic

connectivity. With the demonstration of genetic connectivity and the promise of continued federal and state efforts to address genetic connectivity, wolves were again delisted in the NRM, except in Wyoming as of April 2009 (USFWS 2009). Our study is the first ecosystem-wide assessment of genetic structure and connectivity for the NRM wolf population following their recovery.

Our results showed that high genetic diversity was maintained throughout the first decade of recovery, and genetically effective dispersal was documented between the three recovery areas. These findings demonstrate the success and effectiveness of the re-introduction design and the subsequent protection that promoted rapid population growth. Management for metapopulation dynamics in the NRM will benefit from further knowledge of genetic and demographic parameters in the future, as our results through 2004 are not necessary reliable predictors of future conditions. To this end, managers and biologists could employ periodic sampling of NRM wolves and analyses following methods used here to estimate genetic structure and gene flow. This information could test whether management efforts are facilitating genetically effective dispersal, and such analyses would be required more frequently the smaller the wolf populations. Current wolf management and research programmes in the NRM (see USFWS *et al.* 2010) are conducive to this approach in several ways. First, genetic samples could be collected through existing management frameworks such as regulated hunting or livestock conflict deaths. In 2009, 476 wolves were killed in the NRM through hunter harvest and livestock control. Second, noninvasive sampling techniques, such as faecal sampling, can provide cost-effective, low-intensity population monitoring (e.g. Kohn *et al.* 1999). Third, continued ecological and behavioural studies that involve live capture and radiotelemetry study of individuals can contribute genetic samples as well as information on life history, patterns of reproduction, and movements within and between subpopulations. Although more costly and labour-intensive, information from this monitoring framework will improve predictive population models, and help to identify habitat features important to metapopulation dynamics. Population models that integrate demographic and genetic data are an effective way to assess how demographic parameters (e.g. dispersal rates, effective population size) preserve populations and the genetic variation they contain into the future (Palsbøll *et al.* 2006).

The one migrant per generation rule (Frankel & Soule 1981; Allendorf 1983) has been debated as insufficient because of the simplifying assumptions (e.g. ideal populations whose effective population size equals census size), with an equally debated number of 10 migrants per generation suggested to be more appropriate based on demographic parameters of some natural populations (Mills & Allendorf 1996; Vucetich & Waite 2000; Wang 2004b; Fernández *et al.* 2008; Pérez-Figueroa *et al.* 2009). Given that our minimum estimate of 5.4 effective migrants per generation is likely to be smaller than the actual value, sufficient gene flow from natural dispersal may be occurring at a rate that would counteract the loss of future genetic variation within populations because of the drift. Furthermore, given that our data set ends in 2004, our results are limited in their ability to infer population structure and genetic connectivity for current conditions in the NRM. Based on recent NRM population estimates ($n_{2009} = 1706$ vs. $n_{2004} = 835$ wolves; USFWS *et al.* 2010), and increasing evidence for population expansion and dispersal between recovery areas as inferred from telemetry data (USFWS *et al.* 2010), it is likely that greater gene flow is occurring throughout the region currently. However, the applicability of these conclusions to future conditions requires additional analyses and an explicit model of gene flow that incorporates demographic parameters, management regimes, and future land use changes affecting dispersal throughout the NRM. Additionally, the effect of wolf control and hunting will need to be considered. In 2009, 476 wolves (approximately 21% of population) were killed during agency livestock control and hunter harvest throughout the NRM (USFWS *et al.* 2010). In the future, states intend to increase human-caused mortality to reduce the NRM wolf population to about 1200 wolves or 70% of current levels. Whether or not these much higher rates of annual human-caused mortality will play a significant role on effective dispersal and connectivity could be investigated with explicit metapopulation models and future genetic analysis.

In general, to counteract loss of genetic variation, natural dispersal dynamics should be promoted and anthropogenic factors that might significantly reduce genetic connectivity and effective population size should be mitigated. Only about 30% of the census population was found to contribute to breeding in Yellowstone (vonHoldt *et al.* 2008), suggesting that maintenance of adequate effective population sizes might require managing for higher wolf survival in certain areas and during seasonal dispersal peaks (e.g. prior to the breeding season; Mech & Boitani 2003). This issue may now be more of concern given management plans developed by Montana and Idaho that include higher levels of hunting and lethal removal associated with livestock conflict. Buffer zones around core source populations and reduced hunting quotas within known dispersal corridors are examples of management practices that might help maintain the recent levels of genetically effective dispersal between recovery areas and enhance

natural evolutionary processes and ecological dynamics in large protected areas (Loveridge *et al.* 2007). Translocation events can be considered as a last resort to replace natural migration for subpopulations at risk of isolation, as it was shown here to result in gene flow from two wolves translocated from Montana to Yellowstone that successfully reproduced (vonHoldt *et al.* 2008).

We demonstrate the importance of regional patterns of genetic variation to population management and significantly show how this analysis can be applied to re-introduced populations derived from the same pool of founders. Further, given that few endangered species re-introductions succeed (Griffith *et al.* 1989; Wolf *et al.* 1996; Fischer & Lindenmayer 2000), our study is one of the first to evaluate the genetic consequences of a highly successful re-introduction and consequent population expansion. We found unexpectedly that sibship methods identified migrants and genetically effective dispersal more accurately, and of more individuals than assignment approaches. Wolves have high dispersal capability and demographic resiliency to natural and anthropogenic mortality factors relative to other vertebrate species at risk (Mech & Boitani 2003). Moreover, the NRM has high-quality habitat compared to other areas in the United States. (Carroll *et al.* 2003; Oakleaf *et al.* 2006). However, managers should anticipate much greater difficulty achieving and maintaining wolf restoration in areas with less suitable or more fragmented habitat. Consequently, managing NRM wolves for biological viability while addressing human conflicts in a topographically varied landscape of livestock production, big-game hunting, and relatively high human density still remains a challenge. Although the current NRM wolf population is estimated at 1706 individuals, maintaining the population at or above this level is unlikely based on current agency management plans which intend to reduce the NRM wolf population to about 1200 wolves (USFWS *et al.* 2010). If the NRM wolf population were to be managed below the levels we studied, increased monitoring and management to enhance wolf survival in the NRM could be warranted (Smith *et al.* 2010). As a result, management of wolves near the minimum post-delisting management targets (~450 wolves; USFWS 2009) would be more costly to managers responsible for maintaining adequate population sizes and genetic connectivity, ecological arguments aside. Furthermore, the success of dispersers will decrease as wolf mortality rates by hunting and control for livestock depredations increase, or if habitat outside of core protected areas becomes less suitable because of the land management practices (Oakleaf *et al.* 2006; Murray *et al.* 2010; Smith *et al.* 2010; USFWS *et al.* 2010). Conse-

quently, a management challenge for long-term viability of wolves in the NRM will continue to be the maintenance of adequate population size and effective dispersal to maintain long-term genetic health. Encouraging natural dispersal throughout the NRM and Canada should remain a priority for future conservation of the NRM wolf population.

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Supporting information

Additional supporting information may be found in the online version of this article.

Data S1 Materials and methods.

Fig. S1 Mean and standard deviation bars of Structure's Ln $P(X \mid K)$ for 10 iterations of each K value of the unlinked admixture model for the (A) Founding phase, (B) Colonization phase, and (C) Contemporary phase.

Table S1 Sample size (N), allelic diversity (k), observed/expected heterozygosity (H_O and H_E, respectively), and polymorphic information content (PIC) estimates for the founding phase

Table S2 Sample size (N), allelic diversity (k), observed/expected heterozygosity (H_O and H_E, respectively), and polymorphic information content (PIC) estimates for the colonization phase

Table S3 Sample size (N), allelic diversity (k), observed/expected heterozygosity (H_O and H_E, respectively), and polymorphic information content (PIC) estimates for the contemporary phase

Table S4 Founding phase private alleles and their frequencies (f)

Table S5 Detailed results from migrant detection method for 23 individuals

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