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Developing Metapopulation Connectivity Criteria from Genetic and Habitat Data to Recover the Endangered Mexican Wolf

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Abstract: *Restoring connectivity between fragmented populations is an important tool for alleviating genetic threats to endangered species. Yet recovery plans typically lack quantitative criteria for ensuring such population connectivity. We demonstrate how models that integrate habitat, genetic, and demographic data can be used to develop connectivity criteria for the endangered Mexican wolf (*Canis lupus baileyi*), which is currently being restored to the wild from a captive population descended from 7 founders. We used population viability analysis that incorporated pedigree data to evaluate the relation between connectivity and persistence for a restored Mexican wolf metapopulation of 3 populations of equal size. Decreasing dispersal rates greatly increased extinction risk for small populations (<150–200), especially as dispersal rates dropped below 0.5 genetically effective migrants per generation. We compared observed migration rates in the Northern Rocky Mountains (NRM) wolf metapopulation to 2 habitat-based effective distance metrics, least-cost and resistance distance. We then used effective distance between potential primary core populations in a restored Mexican wolf metapopulation to evaluate potential dispersal rates. Although potential connectivity was lower in the Mexican wolf versus the NRM wolf metapopulation, a connectivity rate of >0.5 genetically effective migrants per generation may be achievable via natural dispersal under current landscape conditions. When sufficient data are available, these methods allow planners to move beyond general aspirational connectivity goals or rules of thumb to develop objective and measurable connectivity criteria that more effectively support species recovery. The shift from simple connectivity rules of thumb to species-specific analyses parallels the previous shift from general minimum-viable-population thresholds to detailed viability modeling in endangered species recovery planning.*

Keywords: *Canis lupus baileyi*, circuit theory, conservation planning, Endangered Species Act, least-cost distance, metapopulations, population viability

Desarrollo de Criterios de Conectividad Metapoblacional a Partir de Datos Genéticos y de Hábitat para Recuperar al Lobo Mexicano en Peligro de Extinción

Resumen: *Restaurar la conectividad entre poblaciones fragmentadas es una herramienta importante para aliviar las amenazas genéticas para las especies en peligro. A pesar de esto, los planes de recuperación típicamente carecen de criterios cuantitativos para asegurar la conectividad de dicha población. Demostramos cómo los modelos que integran los datos de hábitat, genéticos y demográficos pueden ser utilizados para desarrollar criterios de conectividad para el lobo mexicano (*Canis lupus baileyi*) que se encuentra en peligro y actualmente está siendo reintroducido a la vida silvestre a partir de poblaciones cautivas que descienden de 7 fundadores. Usamos el análisis de viabilidad poblacional, que incorporó datos del árbol genealógico, para evaluar la relación entre la conectividad y la persistencia para una metapoblación restaurada de lobo mexicano con 3 poblaciones de igual tamaño. La disminución de las tasas de dispersión aumentó el riesgo de extinción de poblaciones pequeñas (<150-200), especialmente cuando las tasas de dispersión bajaban más allá de 0.5 migrantes genéticamente efectivos por generación. Comparamos tasas de migración observadas en la metapoblación de lobos de las Montañas Rocallosas del Norte con 2 medidas efectivas de distancia*

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basadas en el hábitat, de menor costo y de distancia de resistencia. Después usamos la distancia efectiva entre dos poblaciones potenciales de núcleo primario en una metapoblación reintroducida de lobo mexicano para evaluar las tasas potenciales de dispersión. Aunque la conectividad potencial fue más baja en los lobos mexicanos frente a la metapoblación de lobos de las Rocallosas del Norte, una tasa de conectividad de >0.5 migrantes genéticamente efectivos por generación puede obtenerse por medio de dispersión natural bajo las actuales condiciones de paisaje. Cuando hay suficientes datos disponibles, estos métodos permiten a los planificadores moverse más allá de las metas de conectividad esperadas o de reglas generales para el desarrollo de criterios objetivos y medibles de conectividad que apoyen con mayor eficiencia la recuperación de la especie. El cambio de reglas generales de conectividad simple a análisis específicos de especies es similar al cambio previo de umbrales de mínimos generales de viabilidad de población a modelos detallados de viabilidad en la planificación de la recuperación de especies en peligro.

Palabras Clave: Acta de Especies en Peligro, *Canis lupus baileyi*, distancia de menor costo, metapoblaciones, planificación de conservación, teoría de circuitos, viabilidad poblacional

Introduction

Efforts to recover endangered species increasingly involve measures to ensure population connectivity between core habitat areas to enhance population persistence and maintain evolutionary potential (Lowe & Allendorf 2010). The U.S. Endangered Species Act (ESA) requires that recovery plans define "objective and measurable" recovery criteria that comprehensively address the threats that led to listing of the taxa as threatened or endangered (16 U.S.C. §1533 [f][1][B][ii]). However, recovery plans that mention connectivity typically include only aspirational objectives or general rules of thumb (USFWS 1987). Here, we used a case study of recovery planning for the endangered Mexican wolf (*Canis lupus baileyi*) to demonstrate why quantitative connectivity criteria can form an important element of recovery plans and how such criteria can be developed and implemented.

As descendants of the first wave of colonization of North America by the gray wolf (*Canis lupus*), Mexican wolves represent the most genetically unique New World wolf lineage and one of the most endangered mammals in North America (Vonholdt et al. 2011; Wayne & Hedrick 2011). One population of approximately 75 individuals currently exists in the wild, with approximately 300 additional individuals maintained in captivity (Siminski 2012). Genetic threats are greater for the Mexican wolf than for other wolf subspecies because 7 wild founder individuals were the source of all wolves in both the captive and reintroduced populations (Hedrick & Fredrickson 2008). Negative effects of inbreeding on litter size are evident in captive and wild populations of Mexican wolves (Fredrickson et al. 2007). In other small and isolated wolf populations in Europe and North America, inbreeding accumulation has reduced litter size and increased incidence of skeletal defects (Liberg et al. 2005; Räikkonen et al. 2009). Dispersal of even a single migrant into such inbred populations can dramatically affect genetic structure and population performance (Vilá et al. 2003).

Wolves are among the most vagile of all terrestrial mammals and can disperse over 800 km (Forbes & Boyd 1997). Wolves were historically present throughout their range in the contiguous 48 states as a largely continuous population with some degree of genetic isolation by distance and additional heterogeneity reflecting ecological factors (Vonholdt et al. 2011). Due to habitat loss, overexploitation, and other factors, future wolf distribution in the United States outside of Alaska is likely to consist of many relatively disjunct subpopulations, and these subpopulations will be small relative to historic population sizes (>300,000; Leonard et al. 2005). However, given the species' vagility, achieving connectivity via natural dispersal may be feasible within such a metapopulation. Rigorous assessment of the influence of connectivity as well as population size on viability is thus a necessary component in wolf recovery planning (Wayne & Hedrick 2011).

We demonstrate how results from population viability analyses can be combined with habitat data to develop quantitative recovery criteria for population connectivity. We used population viability analysis (PVA) that incorporated pedigree data to address the relation between connectivity and persistence for the species. Pedigree data for the existing wild population and for new populations founded by hypothetical captive pairings designed to minimize relatedness allowed us to realistically assess the effects of genetic management on restoration success. We then used habitat-based effective-distance metrics to determine the level of natural dispersal feasible given expected management and landscape characteristics. These models also allow identification of specific linkage areas in which connectivity conservation efforts can be focused. When sufficient data are available, these methods allow planners to move beyond general aspirational connectivity goals or rules of thumb to develop objective and measurable connectivity criteria that more effectively support species recovery.

Methods

Context of Case Study

We used information from previously published studies to determine what areas within the southwestern United States and northern Mexico contained sufficient habitat to support populations of Mexican wolves. The majority of the subspecies' historic range occurred in Mexico (Leonard et al. 2005). However, high human-associated mortality risk and low prey density within potential core areas in Mexico suggests that these areas are unlikely to support populations of over 100 individuals (Araiza et al. 2006). Therefore, we also considered potential reintroduction areas in the southwestern United States that were outside the historic range of the Mexican wolf but within the historic zone of genetic intergradation between Mexican wolves and more northerly wolf populations (Leonard et al. 2005). Projections of increasing aridity in the southwestern United States due to climate change (Notaro et al. 2012) suggest that establishment of populations at or beyond the northern extreme of the historic range may be an appropriate strategy to increase metapopulation resilience.

We used a 2-stage process to evaluate potential recovery criteria for the Mexican wolf. Stage 1 consisted of a PVA in which population performance across a range of scenarios was compared with alternative population size and connectivity criteria. In stage 2, we used effective-distance metrics derived from habitat data to evaluate what rates of dispersal could be expected between the reintroduced populations. By combining information from these 2 stages, we were able to evaluate what combination of population size and connectivity criteria allowed recovery of a metapopulation of Mexican wolves given current habitat conditions.

PVA is a structured method of integrating information on diverse threats to a population's persistence. Due to the magnitude of genetic threats to the Mexican wolf, we used an individual-based population simulation model (Vortex) (Lacy 2000; Lacy & Pollak 2012) that allows exploration of how genetic threat factors vary with population size and metapopulation structure. We combined the Vortex results with data from a previously published model (Carroll et al. 2006) that evaluated the distribution of potential wolf habitat in the southwestern United States.

Carroll et al. (2006) used a spatially explicit population model that allowed detailed treatment of spatial population dynamics and habitat configuration but lacked consideration of genetic issues. Their results suggest that the southwestern United States has 3 core areas with long-term capacity to support populations of several hundred wolves each. These 3 areas, each of which contains a core area of public lands subject to conservation mandates, are in eastern Arizona and western New Mexico (i.e., Blue Range, the location of the current wild population), northern Arizona and southern Utah (Grand Canyon), and northern New Mexico and southern Colorado (Southern Rockies) (Carroll et al. 2006). Based on the number and location of potential core areas, we structured our analysis to evaluate performance of a metapopulation of 3 populations and varied population size and connectivity across a range of plausible recovery criteria.

Vortex Simulations of Population Viability

The Vortex model simulates the effects of both deterministic forces and demographic, environmental, and genetic stochastic events on wildlife populations (Lacy 2000; Lacy & Pollak 2012). Vortex simulates a population by stepping through a series of events that describe an annual cycle of a sexually reproducing, diploid organism. Vortex tracks the sex, age, and parentage of each individual in the population as demographic events are simulated. Vortex allows the user to specify the pedigree of the starting population and uses the genetic relationships among founders to derive inbreeding coefficients and other genetic metrics in subsequent simulated generations. Vortex allows tracking of both demographic metrics (population size, time to extinction) and genetic metrics (heterozygosity, allelic diversity, and inbreeding coefficient).

We adapted the Vortex model structure to make it appropriate for analysis of connectivity effects for a species with a complex social breeding system. We incorporated into the model the persistent monopolization of breeding opportunities by male and female alpha individuals. Once an individual achieves alpha status it will generally retain that status until death. This aspect of the wolf social system reduces genetic effective population size (N_e) and thus may enhance inbreeding effects. We also modified Vortex to track the observed number of genetically effective migrants per generation (here termed *migrant* and defined as the total number of individuals from all other populations that produces at least one offspring in the recipient population). These results were used to assess the effects of dispersal on population persistence and inform development of a recovery criterion for population connectivity. Alternative recovery criteria for population size were evaluated by creating a numeric threshold above which a percentage (10–16%) (Table 1) of any surplus individuals were removed annually. Further details, metadata, and sample input files documenting model structure are provided in Supporting Information.

We parameterized Vortex with available information from the wild Mexican wolf population (Fredrickson et al. 2007), the Northern Rocky Mountains (NRM) metapopulation (Smith et al. 2010), and other wolf populations (Supporting Information). We did not base model parameterization solely on data from the existing wild Mexican wolf population for 2 reasons. First, we analyzed potential persistence of populations reintroduced to new areas whose demographic rates may not match those of any extant population. Second, the existing wild population remains heavily manipulated via management removals and re-releases. Human-caused wolf mortalities in the existing wild population constituted 81% of the

Table 1. Results of sensitivity analysis of Vortex population model assessed using standardized coefficients from logistic regression of parameter sets against probability of extinction and quasi extinction.

			z value for probability of		
Parameter	*Minimum*	*Maximum*	*extinction*	*quasi-ex.-150*	*quasi-ex.-250*[a]
Adult mortality[b]	18.32	27.48	167.46	162.48	111.15
Percentage of females in breeding pool	40	60	−160.67	−156.80	−104.49
Population size threshold	50	350	−158.63	−136.53	−72.03
Strength of inbreeding depression[c]	6.586	9.789	152.81	141.54	92.90
Density dependent reproduction	categorical		−92.42	−54.95	−8.35
Effective migrants per generation	0.0	2.4	−88.13	−56.17	−35.49
Average number of years between disease events	4	6	76.54	81.23	41.31
Pup mortality[b]	19.52	29.28	75.37	60.22	43.56
Variation between existing and new populations[d]	categorical		−34.12	−32.62	−24.79
Carrying capacity buffer[e]	1.07	1.60	−5.44	−51.50	−52.47
Harvest efficiency[f]	6.4	9.6	−3.86	−2.44	−12.65

[a] *Quasi-extinction occurs when the 8-year running mean population size falls below 150 or 250. All regressions are based on 1000 scenarios derived from randomized parameter sets, with 100 replicate runs per scenario. Standardized regression coefficients (z values), created by dividing a regression coefficient by its standard error, are unitless values whose magnitude indicates the relative importance of a parameter in the model.*

[b] *From Smith et al. (2010) for Greater Yellowstone Area wolf population.*

[c] *Slope parameter in equation of Fredrickson et al. (2007) relating litter size to inbreeding coefficient.*

[d] *Variation in population performance arising from contrasts between populations in initial pedigree.*

[e] *Ratio of ecological carrying capacity to the population size threshold parameter.*

[f] *Reciprocal of proportion of the population above the population-size threshold that is removed annually.*

mortalities with known causes from 1998 to 2011, primarily due to illegal shooting (43%), vehicle collisions (14%), and lethal management removals (12%) (Turnbull et al. 2013). However, since 2009, when revised management protocols restricted management removals, the wild population has shown positive demographic trends, growing from 42 to 75 individuals (USFWS 2012). Demographic rates in the wild population, particularly survival rate, thus remain highly contingent on management policy regarding removals. Our goal here was not to review the current status of the existing wild Mexican wolf population, but to assess what conditions would allow recovery of the subspecies as a whole.

Analysis of the potential effects of stochastic factors on viability requires the assumption that demographic rates alone will not cause deterministic population decline. However, demographic data collected over the last decade for the wild Mexican wolf population imply an intrinsic population growth rate of <1 (USFWS 2012). We therefore used mortality rates from the wolf population in the Greater Yellowstone Ecosystem (GYE) because mortality rates there (24.4% and 22.9% for pups and nonpups [yearlings and adults], respectively [Smith et al. 2010]) are intermediate among the 3 NRM core populations and represent a plausible goal for mortality rates after recovery actions are implemented but before delisting (Smith et al. 2010). Our baseline demographic parameter set resulted in a deterministic lambda of 1.23, which is similar to that used in previous Mexican wolf PVAs (Seal 1990; IUCN 1996). We evaluated the effect of alternate assumptions concerning mortality rates as part of the sensitivity analysis described below.

All simulated populations were started with wolves produced from the existing Mexican wolf pedigree (Siminski 2012). Founders of the existing wild (Blue Range) population were based on the known 2013 composition of the population projected forward 9 years to a starting population of 122 wolves (Supporting Information). The 2 other simulated populations were founded by assuming 2 pairs would be released each year from 2018 through 2022 into each population. We selected individuals for release from a hypothetical new generation of captive-born wolves that were minimally related and collectively represented genetic variation in the existing captive and wild populations. Released individuals produced offspring and experienced mortality after release, and surviving founders and offspring formed new pairs such that at the start of 2022 each of the 2 new populations contained 50 wolves and 10 pairs (Supporting Information).

Sensitivity Analysis

Although wolves are among the best studied of large mammals, substantial uncertainty exists on how to appropriately parameterize demographic models. We performed a global sensitivity analysis by generating 1000 sets of parameters in which values for 9 key parameters were drawn from a random uniform distribution with a range equal to ±20% of the mean value ("relative sensitivity analysis" [Cross & Beissinger 2001]) from their best estimates (Table 1). We also varied target population size and connectivity rates across a uniform distribution spanning a range of recovery criteria values (Table 1).

Each of the 1000 parameter sets was evaluated based on 100 replicate simulations of 100 years each.

We used a relative sensitivity analysis because several parameters were either aspects of model structure for which empirical distributions do not exist (carrying-capacity buffer [i.e., the proportion by which ecological carrying capacity exceeds the population size parameter] and harvest efficiency [i.e., proportion of the population above the population size parameter that is removed in a particular year]) or would be difficult to derive from the literature (Seal 1990; IUCN 1996) (see Supporting Information for references for demographic parameters in Table 1).

We used standardized coefficients from logistic regression of parameters against extinction and quasi-extinction outcomes to rank the effect of parameters on outcomes (Cross & Beissinger 2001). Dividing a regression coefficient by its standard error results in a standardized regression coefficient or z value, which expresses the unique contribution of that parameter scaled by the variability of the parameter (Cross & Beissinger 2001). The resulting z values (Table 1) are unitless and interpretable only in comparison with other z values in the same model. Significance tests and associated P values would be uninformative because the large number of scenarios considered (1000) arbitrarily inflates sample size.

Following the global sensitivity analysis, we generated 1000 scenarios of parameters in which population size and connectivity rates were again drawn from a random uniform distribution but other parameters were fixed at their mean values (Table 1). We used locally weighted regression (loess) (Cleveland & Devlin 1988) to evaluate in more detail the relation of extinction and quasi extinction to population size and connectivity rate.

Endangered and Threatened Status under the ESA

The ESA defines an endangered species as "at risk of extinction throughout all or a significant portion of its range" (16 U.S.C. §1532[3.6]) and a threatened species as "likely to become endangered in the foreseeable future" (16 U.S.C. §1532[20]). The statute does not provide a quantitative definition of *at risk of extinction*. Recovery plans typically include risk thresholds of 1% to 10% over periods ranging from several decades to a century. There is less agreement over interpretation of the statute's definition of threatened status. Angliss et al. (2002) proposed that, to be consistent with the statute, criteria for threatened status should be defined by reference to the criteria for endangered status rather than directly in terms of extinction risk. This approach was subsequently incorporated into recovery plans for species such as the fin whale (*Balaenoptera physalus*), which will be removed from the list of threatened species when it "has less than a 10% probability of becoming endangered (has more than a 1% chance of extinction in 100 years) in 20 years" (NMFS 2010). We used a time frame for the foreseeable future of 100 rather than 20 years because we analyzed genetic threats that require decades to accumulate to deleterious levels.

Incorporating Multiple Persistence Thresholds

To illustrate how tiered thresholds for endangered and threatened status might be informed by quasi-extinction metrics, we selected a population threshold (150 individuals) that corresponded to adequately low extinction risk ($<10\%$) in exploratory analyses with baseline demographic rates. We then measured the proportion of simulations with a population size criteria of >150 in which, after the initial 30 years of population establishment, the 8-year (2 generation) running mean of population size drops below 150. As with extinction probability, the metrics report the mean quasi-extinction probability across the 3 populations.

Populations of most species continue to increase under state-level management after recovery and removal (delisting) from the federal list of threatened species. However, because wolves can negatively affect other resources (livestock, wild ungulates), state agencies may seek to manage delisted wolf populations at the lowest level consistent with maintaining recovered status. Due to genetic and other issues, long-term management of populations to a harvest-imposed ceiling may result in deterioration in vital rates (Mills 2012). Population thresholds implemented by the states after federal delisting are analogously related to threatened status in that they must ensure an adequately low probability of becoming threatened in the foreseeable future. This risk can be measured by a second quasi-extinction metric based on the probability of population size dropping below the threshold dividing endangered and threatened status (which was developed as described above). Under the ESA's framework, the thresholds that distinguish extinct, endangered, threatened, and recovered species are thus interrelated and can be quantitatively assessed with a unified set of PVA-based metrics.

Feasibility of Alternative Connectivity Criteria

We assessed what rate of natural dispersal between potential core populations could be achieved given the distribution of habitat. We projected connectivity rates between primary core populations in the Mexican wolf metapopulation by relating observed connectivity rates in the NRM metapopulation (Vonholdt et al. 2010) to habitat-based effective distance between populations in both the NRM and the southwestern United States. Because published data on effective migration rate in the NRM are insufficient to build a predictive model, this extrapolation is necessarily qualitative, but nonetheless informative in this planning context. We also compared habitat-based distances between the Mexican wolf and NRM metapopulation with the distances within those metapopulations to evaluate potential dispersal rates between the 2 metapopulations.

We compared results from 2 contrasting effective-distance metrics based on least-cost (shortest-path) distance and resistance (current flow) distance, respectively (Carroll et al. 2012) in order to assess the robustness of conclusions to choice of connectivity metric. Both least-cost distance and resistance distance have been correlated with gene flow in several species (McRae et al. 2008). Habitat suitability index values from a previously published study (Carroll et al. 2006) were assumed to be proportional to movement cost and conductance (see supplementary material S2 for description of habitat model). Least-cost distance, calculated using the Linkage Mapper software (McRae & Kavanagh 2011), represents cost of movement as distance, and identifies the single optimal path between two predetermined endpoints that has the shortest total distance (least total cost). In contrast to least-cost distance, resistance (current flow) distance integrates the contributions of all possible pathways across a landscape or network. We used Circuitscape software to calculate a resistance distance statistic that summarizes overall connectivity between each pair of core areas (McRae et al. 2008). Additionally, Circuitscape produced maps of current flow that can help planners direct conservation measures toward areas important for connectivity.

Results

Effects of Population Size and Connectivity on Extinction and Endangerment

Population size and dispersal rate interacted to influence probability of extinction and quasi extinction (Table 1, Figs. 1 & 2). Dispersal rate strongly affected extinction probability at population criteria below 200 but decreased in importance at larger population sizes (Fig. 1a). Dispersal rates of <0.5 migrants greatly increased extinction risk (Fig. 1b). Extinction risk continued to decrease at rates between 0.5 and 1 migrants for populations of <150, but there was less effect of increased dispersal on persistence for larger population sizes (Fig. 1b).

Dispersal rate had less effect on probability of endangerment (defined here by a quasi-extinction threshold of 150) than on probability of extinction (Table 1). Higher dispersal rates reduced the probability of endangerment in 2 ways. First, and most importantly, higher dispersal rates reduced the population size threshold corresponding to an extinction probability that was adequately low to merit downlisting (Fig. 1a). Second, higher dispersal rates reduced the probability of a downlisted population again dropping below that threshold and becoming endangered in the future (Fig. 2). Connectivity had less influence on persistence at the 250 quasi-extinction threshold (Table 1). Simulation results suggested that a buffer for each population of 50–100 individuals above the delisting threshold was needed to adequately reduce the risk that

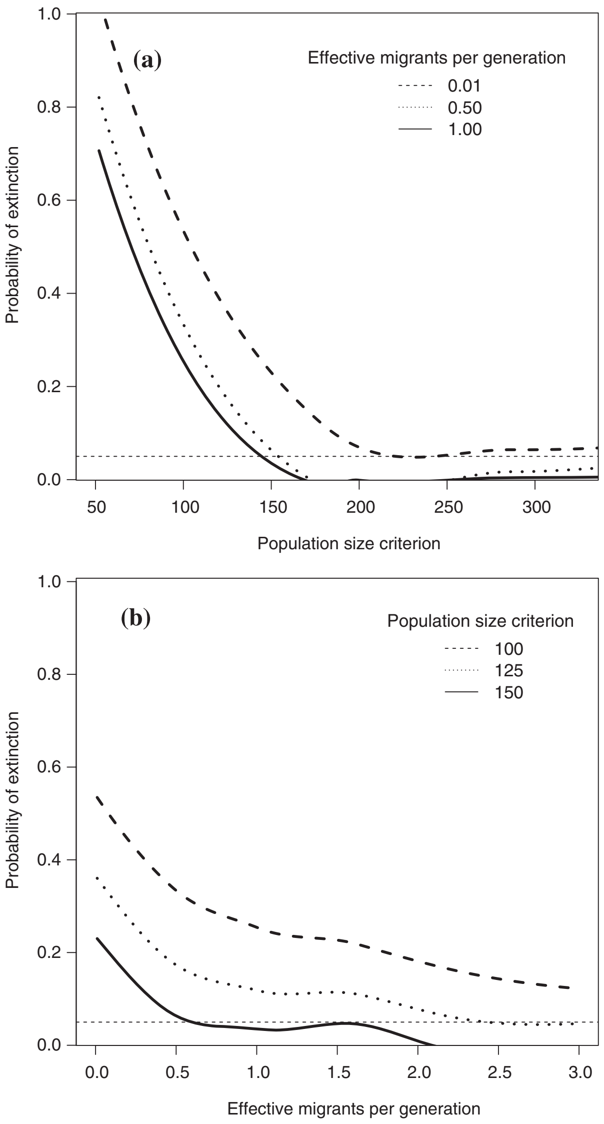

Figure 1. Relation of probability of extinction of Mexican wolf populations to (a) population size criterion and (b) dispersal rate (effective migrants per generation) on the basis of Vortex population simulations of a metapopulation of 3 subpopulations of the specified size. Sensitivity analysis is based on 1000 scenarios derived from randomized combinations of population size and dispersal rate, with 100 replicate runs per scenario. Continuous parameters are set at their mean value and results from categorical variables are averaged. Horizontal dotted line identifies a 5% population extinction-risk threshold commonly used in recovery plans.

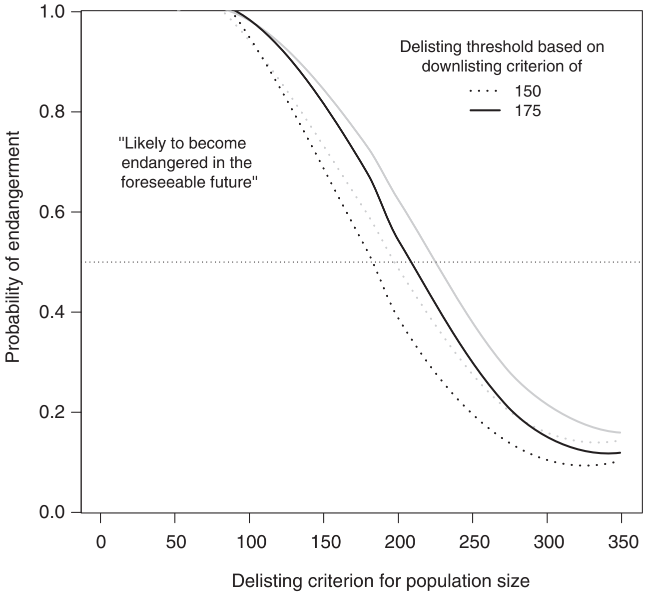

Figure 2. Relation of probability of endangerment (i.e., quasi extinction) to population-size criterion, as derived from the logistic regression of simulation results in the sensitivity analysis (black lines, results given dispersal of 1 effective migrant/generation; grey lines, dispersal of 0.5 effective migrants/ generation). Probability of endangerment is based on the proportion of simulations in which the 8-year running mean of population size drops below a threshold based on analysis of extinction risk (Fig. 1) at any time after year 30 in the simulation. A population is classified as threatened when probability of endangerment exceeds a threshold (e.g., 50%, horizontal dotted line).

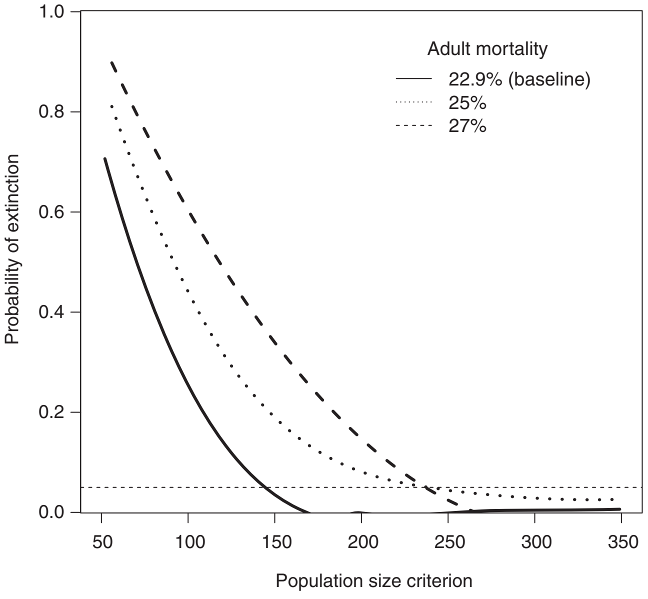

Figure 3. Relation of probability of extinction to population-size criterion under differing levels of adult mortality, as derived from the logistic regression of simulation results in the sensitivity analysis. Dispersal is assumed to be 1 effective migrant/generation. Horizontal dotted line identifies the 5% population extinction-risk threshold.

delisted populations would fall below that threshold in the foreseeable future.

Effects of Demographic Parameters on Persistence and Relisting

Results of the sensitivity analysis suggested that the most important parameters (absolute value of standardized coefficient > 100) were adult mortality, proportion of females in the breeding pool, and strength of inbreeding effects (Table 1). Parameters of intermediate importance (absolute value of standardized coefficient 70–100) were density-dependent reproduction, frequency of disease outbreaks, and pup mortality. Between-population variation was of lower importance. Carrying-capacity buffer and harvest efficiency were the least important parameters. Logistic regression of randomized parameter sets on probability of quasi extinction at either the 150 or 250 population thresholds yielded similar results, except that the effect of the carrying-capacity buffer increased and that of density-dependent reproduction decreased at these larger population thresholds (Table 1). The population-size criterion had as large an effect as the most influential demographic parameters on extinction and on quasi extinction at the 150 threshold but had lower effect at the 250 quasi-extinction threshold. The dispersal-rate criterion was of intermediate importance (Table 1). Conclusions regarding what population-size and connectivity criteria corresponded to a specific extinction risk were contingent on demographic parameters such as adult mortality, which had large z values in the sensitivity analysis (Fig. 3).

Determining Anappropriate Connectivity Criterion

Linkages between primary core populations were ranked similarly (Pearson correlation = 0.85, Spearman rank correlation = 0.72, $n = 9$) under both least-cost distance and resistance distance metrics (Supporting Information). For those linkages ranked more favorably based on resistance distance than based on least-cost distance, multiple linkages may allow more dispersal between those areas than expected based on their single shortest connection (Supporting Information). Projected connectivity between the Blue Range and both the Grand Canyon and Southern Rockies primary core populations was less than that of the 2 best NRM linkages (Supporting Information) but greater than that between the Grand Canyon and

Southern Rockies populations or between Yellowstone and northwestern Montana. When considered in the context of observed NRM migration rates (Hebblewhite et al. 2010; Vonholdt et al. 2010), this comparison suggests that it may be more difficult to achieve a connectivity criterion of 1 migrant/generation for the Mexican wolf in the southwest than for wolves in the NRM.

Because both distance metrics suggest that few direct migrants would be expected between the Grand Canyon and Southern Rockies, we structured the Vortex PVA to assume dispersal would occur along a chain of 3 populations rather than directly between all pairs of populations. This metapopulation structure provides the most dispersal to the centrally located Blue Range population, which otherwise would perform poorly relative to new populations derived from less-related individuals.

Least-cost and resistance distances between the Mexican wolf and NRM metapopulation were greater than any distances within those metapopulations. Mean intermetapopulation resistance distance was 1.23 and 1.34 that of intrametapopulation resistance distance for the NRM and Mexican wolf metapopulations, respectively. Mean intermetapopulation least-cost distance was 2.59 and 1.81 that of intrametapopulation resistance distance for the NRM and Mexican wolf metapopulations, respectively. Current maps suggest that a potential core area in northern Utah could serve as a key stepping stone to enhance connectivity between metapopulations (Fig. 4).

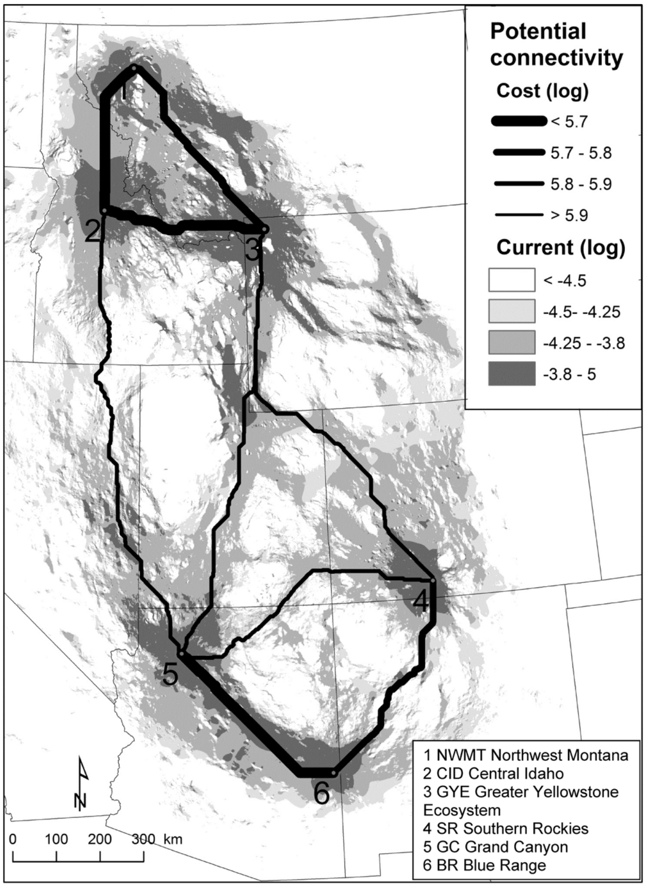

Figure 4. Potential habitat linkages between 6 existing or potential wolf-population core areas in the western United States (thickest lines, linkages with lowest least-cost distance; darkest gray shading, areas with highest importance for connectivity based on the resistance distance model; abbreviations for core areas correspond to labels in Appendix S3 in Supporting Information).

Discussion

Recovery plans for endangered species frequently include either aspirational objectives for maintaining connectivity or general rules of thumb rather than specific quantitative criteria (USFWS 1987). Results from our analysis demonstrate that, where sufficient data exists, quantitative connectivity criteria based on species-specific demographic and habitat data can form an objective and measurable component of recovery plans. Use of pedigree data for the existing wild population, as well as new populations founded by hypothetical captive pairings, allowed us to realistically incorporate genetic effects on restoration success. Results from recent advances in measurement of genetically effective migration rates (Vonholdt et al. 2010) were then integrated with habitat connectivity modeling to predict migration rates and target recovery actions at specific habitat linkages. The shift from simple connectivity rules of thumb to species-specific analyses parallels the previous shift from simple rules of thumb for minimum viable population size to detailed PVA modeling in endangered species recovery planning.

Importance of Connectivity Criteria

Population size had among the strongest influence on population persistence of any parameter evaluated in the sensitivity analysis (Table 1). Connectivity ranked among the moderately important parameters, suggesting that it also merits attention in recovery planning. The importance of connectivity suggested by our PVA results may be most relevant to other species that have been extirpated in the wild and subsequently recovered from a limited number of captive founders or to formerly widespread species that are now limited to small isolated populations. To avoid the genetic damage that may occur during demographic downturns associated with episodic events (e.g., drought, disease), a population derived from inbred and interrelated founders generally must have a larger census population size than a population derived from outbred and unrelated individuals (Allendorf et al.

2012). Similarly, a single effective migrant is more likely to increase persistence of inbred populations (Vilá et al. 2003).

Comparing general rules of thumb on adequate rates of connectivity with results from species-specific simulations can give context to PVA results. The most commonly proposed rule of thumb for connectivity states that one genetically effective migrant per generation into a population is sufficient to minimize the loss of polymorphism and heterozygosity within populations (Allendorf 1983). Our simulation results support use of this rule of thumb because population persistence declined more rapidly at rates below one migrant for smaller populations (<150) (Fig. 1b). Our results also suggest that ensuring lower but nonzero rates of connectivity (e.g., >0.5 migrants) remains important in cases where one migrant may not be achievable. The contrast between our results and previous reviews concluding that a rate of one migrant may be less than optimal for wild populations may be because in our model inbreeding affected persistence solely via effects on litter size, whereas previous reviews considered a broader suite of potential inbreeding effects (Mills & Allendorf 1996). Additionally, we did not consider what population and connectivity criteria would ensure maintenance of adaptive potential through a long-term balance between loss of alleles via genetic drift and new alleles produced by mutation (Franklin & Frankham 1998).

Although wolves are a relatively well-studied species, our simulations necessarily involved substantial uncertainty in both model parameters and structure (e.g., density dependence). Criteria such as population size and connectivity that primarily address stochastic factors remain important even when (as here) effects of deterministic factors and parameter uncertainty are large (Fig. 3). Our baseline parameters were based on the assumption that recovery actions would be effective in reducing the Blue Range population's currently high mortality rates. Alternate mortality-rate parameters would result in different population size and connectivity rates being required to achieve adequate population persistence (Fig. 3). Because metapopulations with adequate connectivity can better withstand less favorable demographic rates, inclusion of a connectivity criterion is precautionary and reduces uncertainty about the future status of a species.

In addition to evaluating extinction probability, we considered 2 quasi-extinction metrics related to probability of relisting as either endangered or threatened. The 2 metrics offered complementary insights regarding the resilience conferred by alternate recovery criteria. An exclusive focus on minimizing extinction might lead to criteria that result in a species persisting in a permanent state of endangerment, which is inconsistent with the intent of the ESA to recover self-sustaining populations (16 U.S.C. §1531[2][b], §1532 [3][3]). Use of multiple persistence metrics focuses attention on the often-ignored genetic and other challenges inherent in managing wildlife populations to a fixed population ceiling (Mills 2012).

Mapping and Managing Population Connectivity

Previous recovery plans for wolves and other large carnivores such as grizzly bears (*Ursus arctos*) noted the importance of metapopulation connectivity but did not develop objective and measurable connectivity criteria (USFWS 1982, 1987). This may have been because at the time such plans were developed, there was less recognition of the synergistic effects of dispersal on genetic diversity and demographic performance of small populations. Due to recent advances in genetic assignment tests and other techniques that allow identification of genetically effective migrants, connectivity is increasingly measurable in wild populations (Vonholdt et al. 2010). When coupled with habitat-based connectivity models, these methods allow development of quantitative connectivity criteria and their incorporation into monitoring programs. Given evidence from other species for utility of effective-distance metrics in predicting gene flow (McRae et al. 2008), they are appropriate tools for informing wolf-recovery planning and demonstrate the utility of applying such methods to data gathered in future monitoring of reintroduced populations. Our results suggest that habitat-based metrics such as least-cost and resistance distance are useful for assessing expected migration rates, but that multiple metrics should be compared to provide a more-informative ranking of alternate linkages.

Differing levels of population connectivity imply qualitatively different genetic effects on populations. We focused primarily on recovery criteria relevant to inbreeding connectivity (Lowe & Allendorf 2010). In this context, our results suggest that viability of the existing wild population is uncertain unless additional populations can be created and linked by dispersal of >0.5 migrants/generation (Fig. 1). In contrast, adaptive connectivity (sensu Lowe & Allendorf 2010) requires only low levels of dispersal (>0.1 migrants) to spread advantageous alleles between populations. Although effective distance metrics suggest that dispersal between the NRM and Mexican wolf metapopulations may be low ($<<0.5$ migrants), this may be sufficient for maintenance of adaptive connectivity, with occasional dispersal maintaining a regional cline in genetic structure similar to historic conditions (Leonard et al. 2005). Recovery plans for formerly widely distributed species should consider how such broad-scale genetic structure can be restored via conservation of interregional linkages and stepping-stone habitat (Franklin & Frankham 1998).

An primary goal of the ESA in seeking to protect threatened and endangered species—as well as the ecosystems on which these species depend—is to recover these species to the point at which they are self-sustaining in their natural habitat (U.S.C. §1531[2(b)]). Preservation of habitat connectivity and necessary levels of natural dispersal is analogous to preservation of the habitat that permits persistence of a wild population of any species. Absent a clear physical barrier to natural genetic exchange (such as a large urban area), achieving connectivity for highly vagile species such as the wolf via natural dispersal rather than artificial translocation is consistent with the intent of the ESA. Integrating PVA and connectivity models as we have done here allows planners to develop such criteria with species-specific PVA and to identify location-specific management actions necessary to meet these criteria and achieve recovery of self-sustaining populations.

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Supporting Information

A description of Vortex model structure and parameters (Appendix S1), description of habitat inputs and methods used in analysis of potential dispersal rates between populations (Appendix S2), plot of resistance distance versus least-cost distance between existing or potential wolf population core areas in the western United States (Appendix S3), and Vortex and connectivity analysis input files showing details of model structure used in simulations (Appendix S4) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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