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Population Structure of Mountain Plover as Determined Using Nuclear Microsatellites

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POPULATION STRUCTURE OF MOUNTAIN PLOVER AS DETERMINED USING NUCLEAR MICROSATELLITES

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Abstract. Mountain Plover (*Charadrius montanus*) is a species of conservation concern that has experienced significant habitat loss and population decline. This, coupled with previous observations that the species exhibits strong fidelity to breeding grounds, suggests that breeding populations may be genetically differentiated and possibly suffer from reduced genetic variation associated with relatively small population sizes. A previous genetic study comparing mitochondrial DNA sequences of plovers in Montana and Colorado found high levels of genetic variability and very little genetic differentiation among breeding locales. Because mitochondrial DNA can track only female movements and is sampled from only one locus, we used 14 nuclear microsatellite loci to further examine population structure, thereby both documenting male movements and providing a more comprehensive view of genetic structure. We found no significant differences among breeding populations. The most likely number of unique genetic clusters was one, suggesting that all sampled breeding locations comprise a single relatively homogenous gene pool. Levels of genetic diversity were similar across all four populations, with the greatest diversity in the southern plains population. We speculate that the lack of detectable genetic differentiation among populations is due to sufficient gene flow among breeding populations that might ensue if at least some pair bonds are formed when birds form mixed flocks on wintering grounds. This study corroborates and expands upon the findings of a previous mitochondrial DNA study providing a more comprehensive view of Mountain Plover population structure.

Key words: *Charadrius montanus*, gene flow, genetic diversity, microsatellites, Mountain Plover, population genetics.

Estructura Poblacional de *Charadrius montanus* Determinada Mediante Microsatélites Nucleares

Resumen. *Charadrius montanus* es una especie que ha sufrido una pérdida significativa de hábitat y una disminución de su población. Este hecho, junto con observaciones previas que han mostrado una fidelidad muy fuerte a las áreas de cría, sugeriría que las poblaciones reproductivas pueden ser genéticamente diferentes y posiblemente sufran de una variación genética reducida asociada con poblaciones relativamente pequeñas. Un estudio genético previo comparó secuencias de ADN mitocondrial y encontró altos niveles de variabilidad genética y muy poca diferenciación genética entre las áreas de cría. Debido a que el ADN mitocondrial solamente puede rastrear los movimientos de las hembras y a que es una muestra de sólo un locus, nosotros utilizamos 14 loci microsatelitales nucleares para examinar la estructura de las poblaciones en más detalle, lo que nos permitió documentar los movimientos de los machos y alcanzar una visión más completa de la estructura genética. No encontramos diferencias significativas entre las poblaciones reproductivas, y el número más probable de grupos genéticos únicos fue uno, lo que sugiere que todas las localidades de cría muestreadas conforman un solo acervo de genes relativamente homogéneo. Los niveles de diversidad genética fueron semejantes a través de cuatro poblaciones, y la diversidad más alta se encontró en la población de southern plains. Especulamos que la falta de diferenciación genética perceptible entre las poblaciones es debida a un flujo de genes suficiente entre las poblaciones reproductivas. Esto es quizás el resultado de la formación de por lo menos algunos vínculos de pareja mientras las aves forman bandadas mixtas en las áreas de invernada. Este estudio corrobora y amplía las conclusiones de un estudio anterior basado en ADN mitocondrial, proporcionando una visión más completa de la estructura poblacional de *C. montanus*.

INTRODUCTION

The Mountain Plover (*Charadrius montanus*) is one of twelve avian species originally thought to be endemic to the grasslands of North America (Mengel 1970). The species is now

believed to be a xeric landscape species that occurs only in disturbed areas of grasslands (Knopf and Wunder 2006), with increased survival rates in times of drought (Wunder 2007, Dinsmore 2008). Mountain Plovers breed from the United States-Canada border south into Mexico, with most birds

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breeding in Wyoming, Colorado, and possibly New Mexico. A small pocket of breeding Mountain Plover occurs in Montana, with isolated birds breeding in Utah, Oklahoma, Kansas, Nebraska, Texas, and Mexico. Although Mountain Plovers winter largely in the Central and Imperial Valleys of California (Knopf and Rupert 1995, Wunder and Knopf 2003, Wunder 2007), some birds winter in Texas and northern Mexico (Knopf and Wunder 2006).

Breeding populations of Mountain Plovers began to decline early in the 20th Century (Laun 1957), with reductions in population sizes across the species' range. In the past 30 years, the rate of population decline has been estimated to be 3% per year (Knopf and Wunder 2006), which has significantly reduced the breeding range of the species. Human expansion into breeding habitat, fire suppression, and changes in agricultural practices as well as livestock and native ungulate grazing regimes have likely been the cause of this population decline (Leachman and Osmundson 1990, Knopf and Wunder 2006). Further, because Mountain Plovers prefer large, open areas with low vegetation and bare ground, they have been positively associated with prairie dog (*Cynomys* spp.) colonies (Dinsmore et al. 2005, Dreitz et al. 2005). As prairie dog colonies have been eliminated by disease outbreaks and intentional extirpation, Mountain Plovers have subsequently suffered population declines (Leachman and Osmundson 1990). Recent continental population estimates for Mountain Plover range from 10 000 to 14 000 birds (Wunder et al. 2003, Plumb et al. 2005), although a new study estimates 10 000 birds in Colorado alone (Tipton 2007). A proposed threatened listing under the Endangered Species Act by the U.S. Fish and Wildlife Service was withdrawn (U.S. Fish and Wildlife Service 2003); however, the Mountain Plover remains a species of high conservation concern.

Adult Mountain Plovers return to the same breeding areas in subsequent years (Graul 1973, Knopf and Wunder 2006). Additionally, some individuals have been documented to return to and breed in the same areas in which they hatched (Dinsmore et al. 2003). This site fidelity, coupled with the declining population sizes, suggests that breeding populations of Mountain Plovers may be differentiated genetically and potentially have relatively low levels of genetic diversity locally (particularly in the smallest and most isolated population in Montana). Data from studies of breeding populations of Mountain Plovers have shown movements among populations in Colorado, yet it is unknown whether the individuals who moved stayed to breed (M. B. Wunder and FLK, unpubl. data). Movement (and subsequent breeding) of even just a few individuals between populations can be enough to offset genetic drift and prevent significant genetic differentiation among populations (Lacy 1987).

A previous genetic study compared mitochondrial DNA sequences of birds from three breeding sites in Colorado and one in Montana to document population structure among

breeding Mountain Plover populations (Oyler-McCance et al. 2005). Those data suggested that the species underwent a population expansion following the Pleistocene glacial period, which likely produced a homogeneous, panmictic group of Mountain Plover 11 000 years ago (Oyler-McCance et al. 2005). Further, the study's authors speculated that there is likely enough current female-mediated gene flow to offset the recent effects of population decline and habitat loss that would predict population differentiation and loss of genetic diversity (Oyler-McCance et al. 2005). Such gene flow might ensue if pair bonds of at least some birds form on wintering grounds where birds from all breeding locales occur in fluid, mixed flocks (Knopf and Rupert 1995, Wunder 2007). These findings are contrary to previous expectations and have important implications for management of the species.

Whereas the use of mitochondrial DNA has its advantages, potential limitations are its maternal mode of inheritance (tracking only female movement) and that it is a single locus. Given the lack of differentiation of the mitochondrial genome found by Oyler-McCance et al. (2005), nuclear markers may show a different pattern of genetic subdivision if dispersal patterns of males and females are dissimilar. Further, the addition of more-rapidly evolving markers sampled across the nuclear genome (many loci) provides finer resolution of differences among populations and better estimates of gene flow among them. This study examines the genetic structure of breeding populations of Mountain Plovers using a suite of highly polymorphic nuclear markers (microsatellites) in order to provide a clearer understanding of population isolation, gene flow, and potential timing for mate choice.

METHODS

MOLECULAR METHODS

We utilized DNA from the 162 blood and embryo samples of the previous mitochondrial DNA study (Oyler-McCance et al. 2005) to examine nuclear microsatellite markers. The four sampling locations comprised the northern plains, central plains, southern plains, and montane breeding populations (Fig. 1). The northern plains population is in southern Phillips County in south-central Montana; the central plains population nests in Weld County, Colorado; the southern plains population comprises birds nesting in Lincoln, Pueblo, and Baca counties in southeastern Colorado as well as Morton County, Kansas; and the montane population is located in a xeric basin surrounded completely by mountain peaks in Park County, Colorado, at a much higher elevation (2600–3500 m; Wunder et al. 2003) than the other breeding locations.

All 162 individuals were screened using 15 nuclear microsatellite loci (*MoPI2*, *MoPI3*, *MoPI5*, *MoPI6*, *MoPI8*, *MoPI9*, *MoPI13*, *MoPI15*, *MoPI17*, *MoPI18*, *MoPI19*, *MoPI21*, *MoPI22*, *MoPI24*, *MoPI26*) isolated from Mountain Plover DNA at the Rocky Mountain Center for Conservation Genetics and

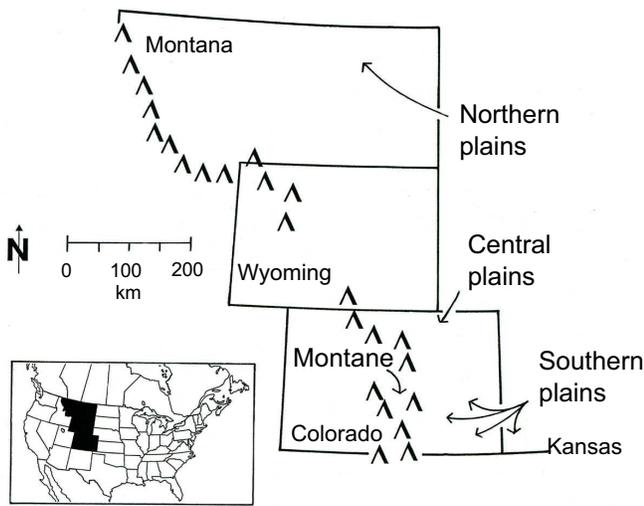


FIGURE 1. Map showing breeding locations of populations of Mountain Plover sampled from 1996–2001 for genetic analysis. The enlarged portion of the figure is from the shaded portion of the inset. The Rocky Mountains are drawn in on this figure to contrast the location of the plains populations and the montane population.

Systematics (St. John et al. 2007). Screening methods and annealing temperatures are outlined by St. John et al. (2007). Each microsatellite locus was amplified using the polymerase chain reaction (PCR) with an M13-tailed forward primer as described by Boutin-Ganache et al. (2001). Each 12.5 μ l PCR contained 125 μ M each dNTP, 1X buffer (Kahn et al. 1998), 0.034 μ M M13-tailed forward primer, 0.5 μ M nontailed reverse primer, 0.31U *Taq* polymerase (Promega, Madison, Wisconsin), and 0.5 μ M M13 dye-labeled primer with Beckman Coulter dyes D2, D3, or D4 (Sigma-Aldrich, St. Louis, Missouri). The thermal profile for the M13 dye-labeled reactions was as follows (the appropriate annealing temperature for each locus is given by St. John et al. [2007]): preheat at 94°C for 1 min, denature at 94°C for 1 min, anneal for 1 min, and extend at 72°C for 1 min. Each PCR had 35 amplification cycles (MJ Research PTC-200, Bio-Rad, Hercules, California). Amplified PCR products were diluted and run on the CEQ8000 XL DNA Analysis System (Beckman Coulter, Fullerton, California). Loci with allele-size fragments less than 400 base pairs were run with the S400 size standard (Beckman Coulter, Fullerton, California) and analyzed with the Frag 3 method of the CEQ Genetic Analysis Software Package (Version 6.0), while those greater than 400 base pairs were run with the S600 size standard and analyzed with the Frag 4 method.

STATISTICAL ANALYSES

Program ARLEQUIN 2.00 (Schneider et al. 2000) was used to test microsatellite genotypes for departures from Hardy-Weinberg equilibrium within each population at each locus.

Linkage disequilibrium was tested for each pair of loci in each population using the program GENEPOP (Raymond and Rousset 1995) with the following parameters: 5000 dememorization steps, 500 batches, and 5000 iterations per batch. The level of genetic diversity in each population was documented by calculating mean unbiased heterozygosity in Genetix 4.05.2 (Belkhir et al. 1996–2004) and mean number of alleles per locus in GenAlEx 6 (Peakall and Smouse 2006). Allelic richness, which adjusts for discrepancies in sample size by incorporating a rarefaction method, was estimated using FSTAT 2.9.3.2 (Goudet 1995).

To investigate population structure, we conducted an analysis of molecular variance (AMOVA) in ARLEQUIN as described by Excoffier et al. (1992). Additionally, pairwise population F_{ST} tests were used to determine whether pairs of populations were significantly different ($P < 0.05$). Program STRUCTURE 2.00 (Pritchard et al. 2000) was used to group individuals without regard to the originating population (employing a model-based clustering analysis) to show any genetic structure and differentiation among sampled populations. We first estimated the number of discrete populations (K) by conducting five independent runs each of $K = 1 - 4$ with 250 000 Markov chain Monte Carlo repetitions, a burn-in period of 500 000 using the model with admixture, correlated allele frequencies, and no prior information. Given the data, the most likely value of K was chosen using the highest likelihood value.

Finally, we estimated directional levels of gene flow and effective population sizes using the maximum likelihood approach of Beerli and Felsenstein (1999) in program Migrate, Version 2.1.3 (Beerli 1997), which is based on coalescent theory. Migrate estimates effective population sizes and the number of migrants per generation by estimating two parameters, Θ and M . The Θ estimate is the product of 4, N_e (effective population size), and μ (the mutation rate), while the M estimate is m/μ , where m represents migration rate (Beerli and Felsenstein 1999, 2001). We can then calculate the number of migrants moving between population i and j per generation ($4Nm_{ij}$) as ΘM and N_e as $N_e = \Theta/4\mu$, with μ = the mutation rate for microsatellites. Since microsatellite loci have been documented to mutate at a rate between 10^{-3} and 10^{-5} mutations per locus per gamete (Dallas 1992, Weber and Wong 1993, Ellegren 1995), we chose to calculate effective population size using both 10^{-4} and 10^{-5} mutations per locus per gamete, realizing that these are rough estimates and that there is evidence that microsatellite mutation rates possibly vary among loci or taxa (Rubinsztein et al. 1995, Ellegren 2000). In Migrate, we used the Brownian motion setting to approximate the stepwise mutation model. The number of trees sampled for the short and long chains were 100 000 and 1 000 000, respectively, and we used adaptive heating with temperatures set to 1, 1.2, 1.5, and 3. We repeated the analysis three times with different random number seeds to assure that we were

TABLE 1. Genetic diversity measures for four breeding populations of Mountain Plover in Montana and Colorado, collected from 1996–2001. Allelic richness is a measure of genetic diversity that corrects for unequal sample sizes.

Sampling locale	Sample size	Mean alleles per locus	SD	Polymorphic loci	Mean observed heterozygosity	Mean expected heterozygosity	Allelic richness
Northern	41	4.07	2.92	14	0.47	0.49	3.78
Central	45	3.64	2.41	13	0.51	0.49	3.56
Southern	41	4.79	3.58	14	0.51	0.49	4.18
Montane	35	4.07	3.25	13	0.47	0.47	3.74

accurately estimating parameters. In the first run, we used F_{ST} values to estimate theta. Subsequent runs used the maximum likelihood estimates from the previous runs to assure that final chains converged on the same estimates (as determined by overlapping 95% confidence intervals).

RESULTS

The number of alleles per locus across all four sampling locales ranged from 1 to 13. One locus (*MoPI3*) was removed from further analysis due to significant deviations from Hardy-Weinberg equilibrium. Of the remaining 14 loci, there were no significant deviations from Hardy-Weinberg equilibrium (Bonferroni corrected $P = 0.0008$). No significant linkages were found between any pairs of loci.

Two populations (central plains and montane) were each found to be monomorphic at one locus (Table 1). The mean number of alleles per locus ranged from 4.79 in the southern plains population to 3.64 in the central plains population. Allelic richness values, which correct for differences in sample size, were similar, with a high of 4.18 in the southern plains population and a low of 3.56 in the central plains population (Table 1).

The results of the AMOVA suggested that essentially no variation could be explained by the among-population designation (–4%) and that all the variation was attributable to within-population variation (104%). Using a Bonferroni-corrected P value of 0.008 to assess significance, pairwise population F_{ST} comparisons revealed that no pairs of populations were significantly different. The most likely number

of discrete genetic clusters determined in the STRUCTURE analysis was one.

The estimates of Θ from Migrate ranged from 0.84 in the montane population to 1.06 in the central and southern plains populations (Table 2). Rough estimates of N_e (due to the uncertainty in the estimate of μ) revealed that the largest effective population size was in the central and southern plains populations and the smallest in the montane population (Table 2). The migration parameter M (which is scaled by μ) ranged from 1.66 to 5.41 (Table 2). The lowest levels of gene flow were generally out of the montane and northern plains populations, and the highest levels were from the central plains population into all other populations (Fig. 2).

DISCUSSION

The levels of genetic diversity were relatively similar among populations yet highest in all measures in the southern plains population, which is consistent with the fact that this sampling locale houses perhaps the largest number of Mountain Plover (U.S. Fish and Wildlife Service 2003) and encompasses the largest amount of area sampled in this study. The remaining three populations had similar levels of genetic variation, with the central plains population having the lowest levels of allelic richness and mean number of alleles per locus, and the northern plains population having the lowest level of observed heterozygosity.

In terms of population structure, we found no significant differences among any of the sampled populations, thereby

TABLE 2. Migrate (Beerli 1997) calculates estimates of migration rate and effective population size using maximum likelihood methods. The parameters estimated for four breeding populations of Mountain Plover in Montana and Colorado, collected from 1996–2001, using program Migrate, are Θ , which is $4*Ne*\mu$ (Ne being the effective population size, with μ being the mutation rate for microsatellite markers) and M , which is the migration parameter equal to m/μ (m being the immigration rate). Profile likelihood estimates (95%) are provided in parentheses. Effective population sizes are presented using two mutation rates for microsatellites, a low estimate using 10^{-4} mutations per locus per gamete as a mutation rate and a high estimate using 10^{-5} mutations per locus per gamete as a mutation rate.

Sampling locale	Θ	Migration parameter (M) into:					
		Ne : low	Ne : high	Northern plains population	Central plains population	Southern plains population	Montane population
Northern	0.99 (0.91–1.08)	249	2485	—	2.16 (1.79–2.52)	1.86 (1.53–2.21)	1.66 (1.29–2.22)
Central	1.06 (0.98–1.13)	266	2659	5.41 (4.61–6.21)	—	4.10 (3.55–4.64)	4.18 (3.58–4.87)
Southern	1.06 (0.98–1.16)	264	2638	2.99 (2.47–3.78)	3.25 (2.82–3.70)	—	3.21 (2.67–3.81)
Montane	0.84 (0.75–0.92)	209	2091	1.97 (1.39–2.46)	1.97 (1.64–2.35)	1.84 (1.51–2.19)	—

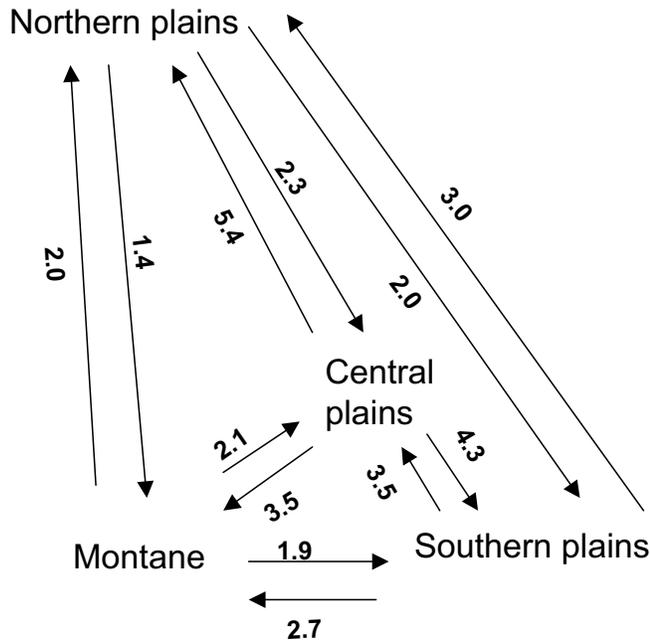


FIGURE 2. Number of Mountain Plover migrants per generation ($4Nm$) moving into and out of each breeding population in Montana and Colorado, sampled between 1996 and 2001. This estimate was calculated as $\Theta * M$.

corroborating the findings of Oyler-McCance et al. (2005) that there is currently enough gene flow among all populations to homogenize the genetic makeup of the breeding populations across the species' range. Our AMOVA results also supported this idea, since essentially no variation could be explained by the between population grouping. The STRUCTURE results also provide evidence of significant genetic exchange among breeding populations, which again is similar to the results found by Oyler-McCance et al. (2005) in their mitochondrial study of the same four breeding populations. Our results are also concordant with a recent study on Snowy Plovers (*C. alexandrinus*) that found little spatial structure among continental populations (Funk et al. 2007), despite that species having a geographic distribution and isolation of breeding locales even greater than that of Mountain Plover.

The estimates of Θ from Migrate in Mountain Plovers ranged from 0.84 in the montane population to 1.06 in the central and southern plains populations. This pattern was similar to the pattern of Θ values estimated by Oyler-McCance et al. (2005) for the same populations using mitochondrial data. This metric can be used to estimate effective population size yet is considered to be a rough estimate due to the uncertainty surrounding estimates of the mutation rates of microsatellite loci (Dallas 1992, Weber and Wong 1993, Ellegren 1995). Our nuclear data suggest that the populations with the largest effective population sizes are the central and southern plains

populations, with almost identical N_e , which is largely consistent with the data from mitochondrial sequencing (Oyler-McCance et al. 2005) study. Further, both studies found that the smallest effective population size occurs in the montane population, contrary to our previous belief that the montane population was one of the larger breeding populations (U.S. Fish and Wildlife Service 2003). A recent stable isotope study (Wunder 2007) further corroborates this finding.

Beerli (2004) discussed how estimates of Θ (and subsequently estimates of N_e) can be biased upwards with the presence of "ghost" populations (i.e., populations nearby that were not sampled in the study). It is possible that the estimates of Θ for the three plains populations of plover could be overestimated due to the fact that our study did not sample all breeding locations that may have been in close proximity to these populations (e.g., breeding Mountain Plover north of the Pawnee National Grassland in Wyoming or in areas north of Phillips County in Montana). In contrast, the montane population is a discrete population surrounded by mountains on all sides, with no effective "ghost" populations in that area.

The number of migrants per generation ($\Theta * M$) was generally lowest out of the montane and the northern plains populations. The highest migration rates were from the central plains population into the southern plains population, which is not too surprising given their close proximity and the relatively minimal geographic barriers between them, and from the central plains population into the northern plains population.

In terms of conservation of Mountain Plover, our data support the assertion of Oyler-McCance et al. (2005) that no single breeding population is genetically unique and that breeding populations have not been genetically isolated from each other for a significant period of time. Further, from a genetic standpoint, no one breeding population requires special conservation attention. Even though the recent history of the Mountain Plover shows pronounced population declines and habitat loss, our data and the data of Oyler-McCance et al. (2005) suggest that levels of gene flow among breeding populations are adequate to maintain comparable levels of genetic diversity in the relatively small populations in this study, which have showed no impact from genetic drift. Thus, despite strong site fidelity to breeding areas, there appears to be sufficient gene flow among sampled populations to negate the factors associated with small population size, genetic drift, and breeding fidelity that lead to population differentiation.

Although the levels of gene flow recorded here and by Oyler-McCance et al. (2005) could be the result of adults changing breeding locations in subsequent years, this is unlikely in this species (Knopf and Wunder 2006), because the breeding locations are geographically distant. We speculate that some juveniles are dispersing from their natal areas and breeding in new locations. Other studies of migratory birds have shown that pairs can form during spring migration or on the wintering grounds (Cooke et al. 1975), and Mountain

Plovers from across the breeding range intermix on wintering grounds (Wunder 2007) where some younger birds likely form pair bonds before migrating to breeding locales.

In summary, our data are consistent with the more preliminary data of Oyler-McCance et al. (2005), who suggested that postglacial population expansion has resulted in a historical increase in genetic diversity, which likely produced a homogeneous, panmictic group of Mountain Plovers. Our more comprehensive view of population structure of Mountain Plovers suggests that although recent population declines and habitat loss have reduced populations of the species, there is sufficient gene flow among breeding areas to mitigate the effects of small population sizes and adult fidelity to breeding areas.

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