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Assessing changes in functional connectivity in a desert bighorn sheep metapopulation after two generations

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Abstract

Determining how species move across complex and fragmented landscapes and interact with human-made barriers is a major research focus in conservation. Studies estimating functional connectivity from movement, dispersal or gene flow usually rely on a single study period and rarely consider variation over time. We contrasted genetic structure and gene flow across barriers for a metapopulation of desert bighorn sheep (*Ovis canadensis nelsoni*) using genotypes collected 2000–2003 and 2013–2015. Based on the recently observed but unexpected spread of a respiratory pathogen across an interstate highway previously identified as a barrier to gene flow, we hypothesized that bighorn sheep changed how they interacted with that barrier, and that shifts in metapopulation structure influenced gene flow, genetic diversity and connectivity. Population assignment tests, genetic structure and genetic recapture demonstrated that bighorn sheep crossed the interstate highway in at least one location in 2013–2015, sharply reducing genetic structure between two populations, but supported conclusions of an earlier study that such crossings were very infrequent or unknown in 2000–2003. A recently expanded population established new links and caused decreases in genetic structure among multiple populations. Genetic diversity showed only slight increases in populations linked by new connections. Genetic structure and assignments revealed other previously undetected changes in movements and distribution, but much was consistent. Thus, we observed changes in both structural and functional connectivity over just two generations, but only in specific locations. Movement patterns of species should be revisited periodically to enable informed management, particularly in dynamic and fragmented systems.

KEYWORDS
dispersal, genetic monitoring, habitat fragmentation, roads

1 | INTRODUCTION

Determining functional connectivity, or how species move through landscapes (Rudnick et al., 2012), has been a major focus in landscape ecology (Betts, Gutzwiller, Smith, Robinson, & Hadley, 2015) and landscape genetics (Manel & Holderegger, 2013). Empirical estimates of functional connectivity are vital for effective management of species in the face of habitat fragmentation and climate change (Creech, Epps, Monello, & Wehausen, 2014; Knowlton & Graham, 2010). In combination with assessments of structural connectivity, determining how species interact with barriers and move across fragmented landscapes has improved the ability to mitigate the impact of such landscape features on wildlife (Clevenger & Waltho, 2005). To investigate whether, and where, individuals cross barriers or human-modified habitats, researchers have employed radiotelemetry, GPS collars generating high-resolution spatial data, behavioural experiments (Moriarty et al., 2015) and remote cameras at potential crossing points (Gagnon, Dodd, Ogren, & Schweinsburg, 2011).

Landscape or population genetic approaches are also widely used for inferring functional connectivity, particularly where species are small-bodied and difficult to monitor with telemetry (Spear & Storfer, 2008), dispersal or long-distance movements are thought to be rare (Davis, Murray, Fitzpatrick, Brown, & Paxton, 2010) or studies encompass large landscapes (Cushman, McKelvey, Hayden, & Schwartz, 2006; Epps, Wehausen, Bleich, Torres, & Brashares, 2007). Both GPS collar and landscape genetic data have served as the basis for developing connectivity or movement models (Chetkiewicz & Boyce, 2009; Creech et al., 2014). Such models have proved fundamental for managing species on fragmented landscapes (Hilty, Lidicker, & Merenlender, 2006) and are preferred for predicting linkages among habitat patches (Rudnick et al., 2012).

Studies aimed at understanding interactions with barriers or animal movement in general are, however, often based on a "snapshot" of patterns on a particular landscape over a few years. Movement models based on direct observation of animal movements, as by GPS telemetry, usually reflect 2–6 years of data (Kertson, Spencer, Marzluff, Hepinstall-Cymerman, & Grue, 2011). Genetic patterns integrate movements over longer and variable timescales (Epps & Keyghobadi, 2015), but genetic investigations of the effects of barriers or fragmented landscapes are almost always based on a single estimate of genetic structure. The stability of patterns and processes inferred from any empirical movement analysis is rarely considered, yet movement or dispersal behaviours themselves may vary over time due to changes in factors such as resource availability (Bowler & Benton, 2005, 2009), parasite load (Debeffe et al., 2014) or population density (Plumb, White, Coughenour, & Wallen, 2009). Thus, models generated in a particular place and time might not capture behaviours under different conditions or newly learned behaviours. Although some studies compare models of movement and connectivity derived from different types of data, very few studies appear to have examined changes in movements or movement behaviours on decadal timescales using the same type of data.

Desert bighorn sheep (*Ovis canadensis nelsoni*) in the Mojave Desert of California are a case study of a species experiencing both natural and anthropogenic habitat fragmentation. Bighorn sheep in this region exist in metapopulations (Bleich, Wehausen, & Holl, 1990; Schwartz, Bleich, & Holl, 1986), with local populations of <25–250 individuals that experience frequent extinction and colonization events (Abella et al., 2011; Epps, McCullough, Wehausen, Bleich, & Rechel, 2004; Epps, Wehausen, Palsboll, & McCullough, 2010). Populations occur in small, sometimes isolated mountain ranges separated by desert flats and bajadas (alluvial fans), as well as fenced interstate highways and other potential anthropogenic barriers (Bleich, Wehausen, Ramey, & Rechel, 1996). Systematic investigation of population genetic structure from 2000 to 2003 and a review of known intermountain movements revealed that gene flow and thus movement of individuals between populations was strongly influenced by distance and topography, and that fenced interstate highways appeared to act as complete barriers (Epps et al., 2005, 2007). Subsequent investigations have treated such barriers as impermeable (Creech et al., 2014). Yet, in 2013, roughly two bighorn sheep generations (assuming 6 years/generation, Coltman et al., 2003) after the 2000–2003 study, an outbreak of respiratory disease associated with the respiratory pathogen *Mycoplasma ovipneumoniae* (Besser et al., 2008), hereafter *M. ovi*, was detected in the Old Dad Peak population in the central Mojave Desert. Several months later, the same strain was detected south of Interstate 40 in the Marble Mountains (T. Besser, Washington State University, and California Department of Fish and Wildlife [CDFW], unpublished data), suggesting stepwise contact by bighorn sheep had occurred across intervening regions, including across the interstate. Avenues for such crossing could include pushing through fencing and crossing at surface level, despite heavy traffic, or using washes bridged by the interstates but also fenced and typically occurring on flatter ground rarely used by bighorn sheep. While transmission of respiratory disease can occur through contact with even a single individual (Besser et al., 2014), this observation raised questions of considerable import for conservation of these metapopulations. Specifically: (i) did bighorn sheep begin crossing barriers within the last two generations, or alternately, (ii) did the spread of the disease indicate that previous genetic analyses were unable to detect ongoing but occasional movements across barriers? Additionally, how dynamic are estimates of genetic structure and genetic diversity across time points?

In this study, we contrast population genetic structure in a dynamic desert bighorn sheep metapopulation across two generations. By sampling the same populations ~12 years apart with the same genetic markers, we attempt to determine whether the interaction of this large mammal with anthropogenic barriers has changed, evaluate the degree of change in genetic structure and genetic diversity across populations and infer sources of recently recolonized or expanded populations. We hypothesized that changes in interpopulation movement patterns of bighorn sheep have occurred since the 2000–2003 study, including new connections formed by expanding populations and crossing of anthropogenic barriers, leading to changes in both structural and functional connectivity in localized portions of the study area. Specifically, we predicted that populations separated by Interstate 40 would show decreased genetic differentiation in 2013–2015 compared to 2000–2003. We also predicted that some individuals would be fully or partly assigned genetically to populations on the other side of the interstate barrier in 2013–2015, but not during 2000–2003, indicating that cross-interstate movements were rarer or undetected at the earlier time, and that pattern would be reflected in first-generation migrants as well. We further predicted that recently established populations in two locations would increase high gene flow linkages among populations. Finally, we consider the implications of this study for studies assessing functional connectivity at a single point in time.

2 | METHODS

2.1 | Study area

This study took place in the southern Mojave and central Mojave Desert metapopulations of desert bighorn sheep (Torres, Bleich, &

Wehausen, 1994) in southeastern California (Figure 1). Those populations were genetically sampled in 2000–2003 (hereafter, Time Point 1 or TP1) by Epps et al. (2005), Epps, Palsboll, Wehausen, Roderick, and McCullough (2006). In 2013–2015 (hereafter, Time Point 2, or TP2), we resampled 13 populations in the core of the Epps et al. (2005, 2006) study area. This sampling area was centred on the recent respiratory disease outbreak first detected at Old Dad Peak in Mojave National Preserve in 2013 (CDFW, unpublished data), as well as one apparently newly colonized population in the South Soda Mountains (Figure 1; Abella et al., 2011). Populations in the resurvey spanned a gradient of genetic diversity and isolation at TP1 (Epps et al., 2005). Interstate 40, a four-lane divided highway fenced on both sides, separated four southern populations from the remainder of the bighorn sheep populations considered in this study (Figure 1). All populations in the study area were native (i.e., never augmented by translocation), except that bighorn sheep from Old Dad Peak were translocated to the nearby North Bristol population in 1992 to mitigate an apparent population extinction in the mid-20th century (Wehausen, 1999). However, by the time of the sampling at TP1, apparently only a few transient males remained (Epps, Bleich, Wehausen, & Torres, 2003).

2.2 | Genetic sampling

We used faecal samples as a primary source of DNA in TP2, collected by visiting water sources during summer months when bighorn sheep are dependent on water and collecting opportunistically at other times of the year. We sampled at the same locations as in Epps et al. (2005) and collected faecal samples up to several weeks in age; if wet, samples were dried before storing at room temperature. We processed pellets and extracted DNA using a modified version of the AquaGenomic Stool and Soil protocol (Multitarget Pharmaceuticals

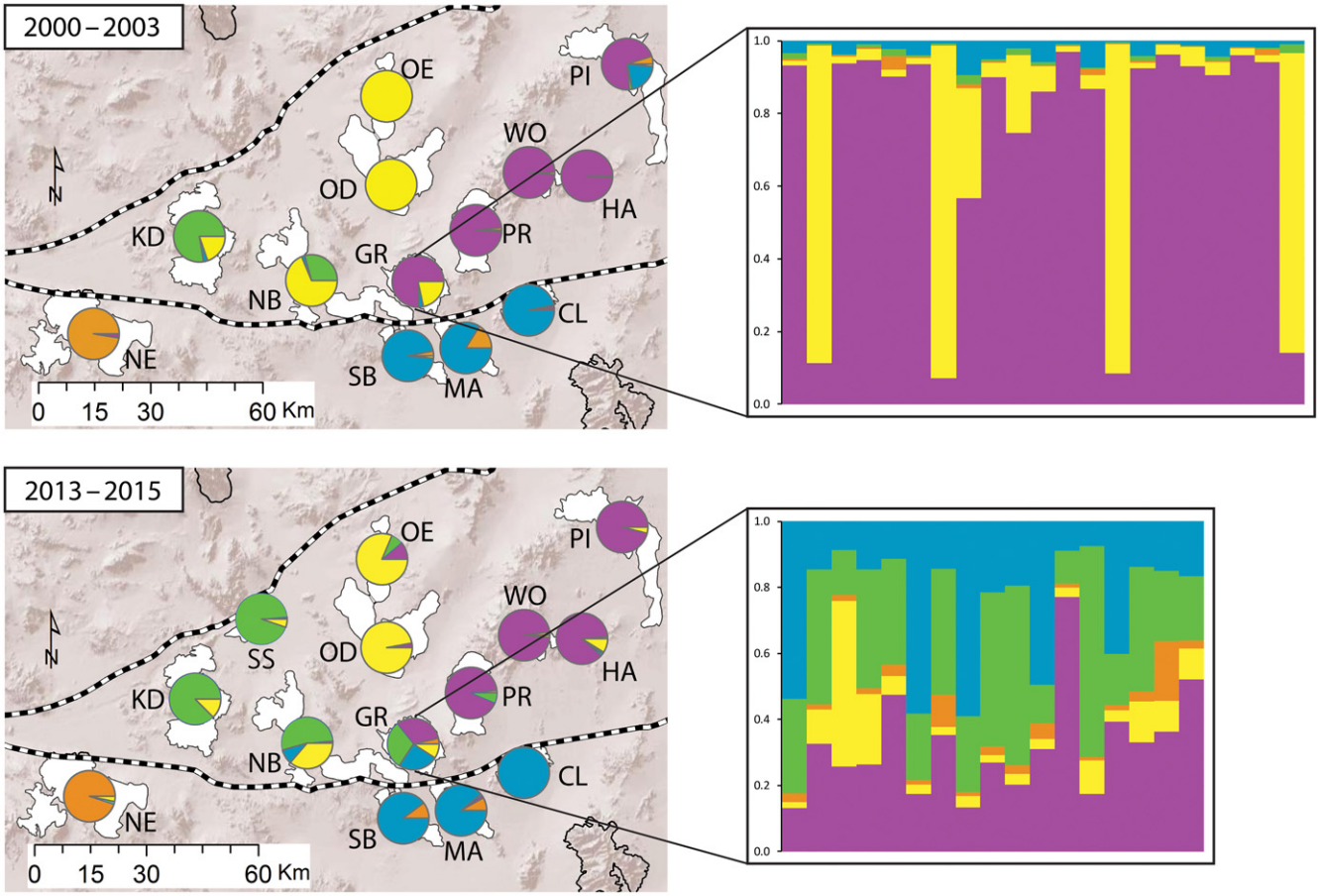

FIGURE 1 Desert bighorn sheep populations genetically sampled at two time points (2000–2003 and 2013–2015, white polygons) in the Mojave Desert of California, with other nearby populations drawn with black outlines, and shaded topographic relief. The South Soda Mountains population, an apparent recent colonization, was sampled only in 2013–2015. Interstate highways are depicted with dashed lines. Average assignments of individuals from desert bighorn sheep populations in 2000–2003 and 2013–2015 ($k = 5$) from Program STRUCTURE are shown colour-coded by proportional assignment to cluster by population (circles) and by individual (Granite Mountains [GR], where each vertical bar reflects an individual). In 2000–2003, no individuals bordering I-40 were assigned to populations on the opposite side, whereas in 2013–2015, five individuals in the Granite Mountains were at least 40% assigned to the populations south of I-40 (blue cluster). Individual assignments for all populations are presented in Figure S3. CL, Clipper Mountains; GR, Granite Mountains; HA, Hackberry Mountains; KD, Cady Mountains; MA, Marble Mountains; NB, North Bristol Mountains; NE, Newberry/Ord/Rodman Mountains; OD, Old Dad Peak/Marl/Kelso Mountains; OE, Indian Spring/Club Peak; PI, Piute Range; PR, Providence Range; SS, South Soda Mountains; WO, Wood Mountains. Polygons modified from Creech et al. (2014)

LLC, Colorado Springs, CO; see details in Appendix S1). We also used DNA extracted from blood of 159 bighorn sheep captured as part of an ongoing demographic study (2013–2015). Capture protocols were approved by the National Park Service IACUC (ACUP #PWR_MOJA_Epps.Powers DesertBighorn_2013). Whole blood was collected in EDTA tubes and spun at 4,000×*g* for 10 min to separate the buffy coat. In 16 cases, DNA was also obtained from ear tips removed from carcasses. We extracted DNA using a Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc, Valencia, CA, USA) and 30 mg of dried tissue or 200 μl of buffy coat.

2.3 | Genotyping, markers, individual identification and marker evaluation

We used 16 variable microsatellite loci to characterize genetic diversity and genetic structure at both time points (Table S1; Appendix S1). Samples at TP1 were genotyped by Epps et al. (2005, 10 loci) and Nickerson (2014, remaining 6 loci). We checked consistency of allele size identification for markers used at both time points by rerunning 16 individuals (to provide a wide diversity of allele sizes) selected across 12 populations from TP1 under laboratory conditions used in TP2 analyses, determining appropriate size corrections, and correcting allele sizes to match those in TP2. Reaction conditions and thermocycling profiles for PCR, genotyping methods, genotype matching and testing for Hardy–Weinberg equilibrium and linkage disequilibrium are described in Appendix S1.

Three of the microsatellite markers were linked to genes related to immune system function in other bovids (BL4, associated with the interferon gamma gene involved in parasite resistance; Coltman, Wilson, Pilkington, Stear, & Pemberton, 2001, TGLA387, linked to the MHC gene complex; Maddox et al., 2001, and TCRBV62, linked to genes for T-cell receptors; Buitkamp, Schwaiger, & Epplen, 1993), but have also been employed as neutral microsatellite markers in systems where they exhibited no evidence of selection (Johnson, Mills, Wehausen, Stephenson, & Luikart, 2011; Luikart et al., 2011). Therefore, we used LOSITAN (Antao, Lopes, Lopes, Beja-Pereira, & Luikart, 2008; Beaumont & Nichols, 1996) to test all microsatellites for positive and balancing selection within each time point. We conducted tests using both stepwise and infinite allele mutation models, using 1,000,000 iterations, approximated mean neutral F_{ST} by removing potential selected loci (Antao et al., 2008) and allowed LOSITAN to select the subsample size for each test. We computed 99% confidence intervals for neutral expectations; loci falling outside those intervals were considered to be potentially influenced by natural selection (Luikart et al., 2011). Because markers under selection can enhance assignment of individuals to source populations (Ogden & Linacre, 2015), all markers were retained for STRUCTURE and GENECLASS analyses. For estimates of genetic structure (F_{ST}), however, we removed markers showing evidence of positive or balancing selection across both time points (Luikart et al., 2011).

After identifying and discarding duplicate individuals and generating complete genotypes for each population (Appendix S1), we used CERVUS (Marshall, Slate, Kruuk, & Pemberton, 1998) to test for matching genotypes across populations. If a genotype was recaptured in more than one population within a time point, for subsequent analyses, we used each genotype only in the population in which it was first detected. Because desert bighorn sheep in this area can live up to ~20 years (J. Wehausen, personal communication, November 21, 2016), we also tested for matching genotypes between the data sets from the two time points. We recorded any such matches but retained matching genotypes in data sets for both time points.

2.4 | Assessing changes in genetic structure and detecting migrants

To ascertain changes in connectivity, including whether bighorn sheep moved across Interstate 40 at either time point, we used genetic recapture (above), estimates of genetic structure, assignment tests and tests for first-generation migrants (i.e., F0, Paetkau, Slade, Burden, & Estoup, 2004; hereafter referred to as migrants). For genetic structure, after removing loci with evidence of selection at both time points (Appendix S1), we used FSTAT (Goudet, 1995) to estimate pairwise F_{ST} (Weir & Cockerham, 1984) between all populations at each time point and estimated 95% confidence intervals by bootstrapping across loci for comparisons of interest. We subtracted pairwise F_{ST} values at TP1 from those at TP2 (hereafter, ΔF_{ST}) to rank changes in genetic structure among populations and compared high gene flow linkages ($F_{ST} \leq 0.05$, Epps et al., 2010) at both time points as an index of meaningful changes in patterns of connectivity. Further, we evaluated pairwise F_{ST} for each population to itself between time points to estimate within-population genetic changes, using 1,000 permutations over loci in ARLEQUIN (Schneider, Roessli, & Excoffier, 2000) to assess difference from zero. To further evaluate potential error in F_{ST} estimates resulting from variation in sample size, we selected three populations representing a gradient of low to high genetic structure and randomly subsampled individuals over a range of sample sizes, estimating pairwise F_{ST} and generating 95% quantiles from 5,000 replicates at each sample size increment (see Figure S1 for full description).

We used STRUCTURE (Pritchard, Stephens, & Donnelly, 2000) to infer individual assignments at both time points in a single analysis combining all data at both time steps, using all loci including any under selection. We used this approach to reduce impact of variation in sample sizes within populations across time steps. We examined individual assignments (q values for each individual to each cluster) within each time point to infer presence of migrants or offspring of migrants among clusters, including across Interstate 40, after estimating assignments (detailed in Appendix S1).

We used GENECLASS2 (Piry et al., 2004) to test for migrants among all populations at each time point, including those separated by Interstate 40. We used all loci including any under selection and applied the Paetkau, Calvert, Stirling, and Strobeck (1995) frequency-based criterion for likelihood computations and a default frequency for missing alleles of 0.01. To estimate the probability of each individual being a migrant, we employed the Paetkau et al. (2004) resampling algorithm, 10,000 simulated individuals, and a threshold significance of $p < .01$.

2.5 | Assessing changes in genetic diversity

We estimated genetic diversity using FSTAT (expected heterozygosity, H_e; average allelic richness, corrected for minimum sample size across loci and time points, A_r) in all populations at TP1 and TP2, using only loci showing no evidence of selection at both time points. For estimating A_r, we further excluded one locus that largely failed to amplify in one small population (see Section 3). After identifying populations of specific interest for genetic diversity comparisons (Marble Mountains and Granite Mountains, see Section 3), we re-estimated H_e and A_r for those populations alone to remove sample size constraints imposed by other populations. Finally, in each of those populations of interest, we tested whether genetic diversity was higher in TP2 than TP1 using paired one-tailed Wilcoxon ranked sum tests on corrected A_r and H_e, implemented in JMP Pro (Version 12.0.1, SAS Institute Inc., ©2015). This test allows comparison of genetic diversity within loci (Luikart et al., 2011).

3 | RESULTS

3.1 | Genotyping, markers and individual identification

In TP2, samples were collected successfully at similar locations as those in TP1 except in the Piute Range, where the concentration of bighorn sheep appeared to have shifted ~30 km from the Viceroy Mine at Hart Mountain in 2003 to Piute Spring in 2015. We generated microsatellite genotypes for 206 unique individuals in 13 populations at TP1 (<4% of all allele calls missing) and 384 unique individuals in 14 populations at TP2 (Table 1; <1% allele calls missing). We detected potential positive selection (using $\alpha = 0.01$) on adaptive-linked microsatellite BL4 at TP1, and BL4 approached significant positive selection at TP2 (Table S2). At TP2, putatively neutral microsatellite OarFCB11 exhibited potential positive selection (Table S2), although this locus did not approach significance at TP1. To create data sets as parallel as possible across time points, we chose to eliminate BL4 from analyses of pairwise F_{ST} and genetic diversity given that it was adaptive-linked and was potentially under or nearly under positive selection at both time points, as well as out of HWE at TP1 (Appendix S1). We did not exclude OarFCB11 from either data set. Excepting BL4, we found no consistent evidence of any locus out of HWE or in linkage disequilibrium (Appendix S1).

Power for determining recaptures of individuals among populations and time points was high: probability of identity (P_{ID}; Waits, Luikart, & Taberlet, 2001) for the full 16-locus data set was 1.02×10^{-13}–2.00×10^{-8} (median 1.18×10^{-11}); P_{IDsibs} was 3.25×10^{-6}–2.47×10^{-4} (median: 1.64×10^{-5}). Genotype matching revealed that bighorn sheep made intermountain movements at both time points (Figure 2; Appendix S1), but crossed an interstate only at TP2: a male first detected from a faecal sample collected in the Granite Mountains in August 2014 was captured in November 2014 in the Marble Mountains on the other side of Interstate 40. Matches also occurred between time points: 5 of 384 unique genotypes sampled at TP2-matched genotypes from TP1 (Appendix S1).

TABLE 1 Population size classes and numbers of genotypes included in population genetic study of desert bighorn sheep in the Mojave Desert, California, at two time points (2000–2003 and 2013–2015)

Population	Genotyped individuals (2000–2003)	Population size-class estimate (c. 2004)	Genotyped individuals (2013–2015)	Population size-class estimate (c. 2010)
CL	16	25–50	34	No update
GR	21	25–50	17	No update
HA	13	25–50 (including WO)	11	No update
KD	12	25–50	20	201–300
MA[a]	29	101–150	47 (46)	151–200
NB	6	0 (transient males only)[b]	50	51–100
NE	14	51–100	25	151–200[c]
OD	25	201–300	48	No update[d]
OE	12	25–50	14	No update
PI	13	51–100	12	No update
PR	20	51–100	26	No update
SB	14	101–150	45	No update
SS	NA	Not known to exist	26	25–50
[a]WO	10	25–50 (including HA)	11 (10)	No update
[a]Total	206	–	386 (384)	–

[a]Sample size in 2013–2015 analyses was subsequently reduced by 1 for MA and WO because one individual in each case was first detected in a different population (GR and HA, respectively).
[b]Bighorn sheep were translocated from Old Dad Peak to North Bristol Mountains in 1992, but the translocation is thought to have failed.
[c]2016 aerial survey by CDFW, unpublished data, based on minimum count.
[d]Thought to have declined sharply in 2013 due to an all-ages die-off from respiratory disease.

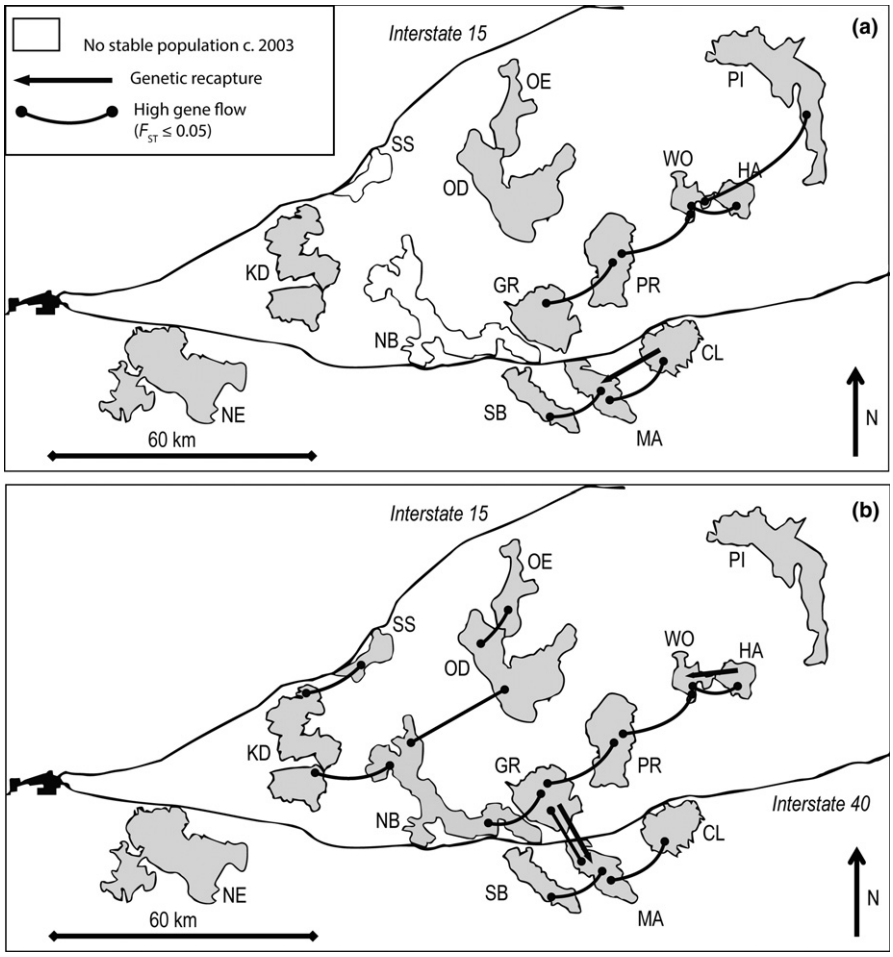

FIGURE 2 Changes in high gene flow linkages (F_{ST} < 0.05, determined by Epps et al., (2010) to be correlated with frequent movements among populations) and genetic recaptures in 2000–2003 (a) and 2013–2015 (b) for desert bighorn sheep populations in the Mojave Desert of California. Arrows represent genetic recaptures between populations within each time point, where the head of the arrow indicates the second observation of that individual. CL, Clipper Mountains; GR, Granite Mountains; HA, Hackberry Mountains; KD, Cady Mountains; MA, Marble Mountains; NB, North Bristol Mountains; NE, Newberry/Ord/Rodman Mountains; OD, Old Dad Peak/Marl/Kelso Mountains; OE, Indian Spring/Club Peak; PI, Piute Range; PR, Providence Range; SS, South Soda Mountains; WO, Wood Mountains

3.2 | Assessing changes in genetic structure and detecting migrants

Population pairwise F_{ST} estimates within the same population between time periods (hereafter, $F_{ST\_TP1:TP2}$) varied ($F_{ST\_TP1:TP2}$ = 0–0.096, Figure 3). Thus, genetic make-up of some populations changed markedly between the two sampling points, as did genetic distances between some pairs of population (Figure 4; Table S3, S4), although many comparisons showed little evidence of change across time points. Median and average genetic distances between the North Bristol Mountains and all other populations decreased the most (median: ΔF_{ST} of −0.041; Figure 4a; Table S4); genetic distances increased the most for comparisons including the Piute Range (median ΔF_{ST} of 0.053; Figure 4b; Table S4). Bootstrapping across loci suggested our power to detect differences in F_{ST} was somewhat compromised by small sample sizes (Figure 4), but experimental variation in sample size while holding loci the same resulted in much less variation in F_{ST} estimates (Figure S1). Genetic distance between populations separated by Interstate 40 declined sharply in at least one case: point estimates of F_{ST} declined from 0.11 to 0.04 (ΔF_{ST} = −0.067) between the Marble and Granite Mountains populations, and neither point estimate intersected the 95% confidence intervals of F_{ST} for the other time period (Table 2; Figure 4c). Other cross-interstate comparisons changed little (ΔF_{ST} = −0.027 to 0.034, Table 2).

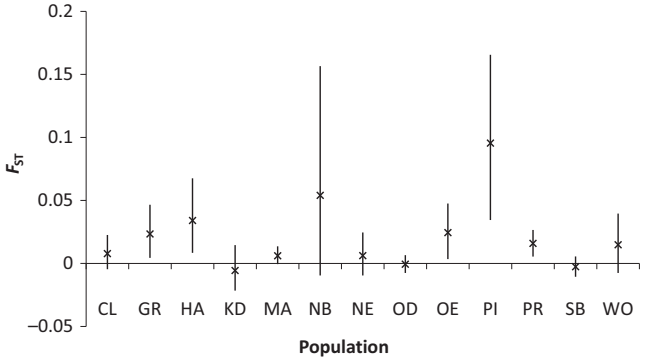

FIGURE 3 Within-population pairwise F_{ST} estimates (crosses) between sampling periods (2000–2003 and 2013–2015) in 13 populations of desert bighorn sheep in the Mojave Desert of California, from 15 microsatellite loci, with 95% confidence intervals. CL, Clipper Mountains; GR, Granite Mountains; HA, Hackberry Mountains; KD, Cady Mountains; MA, Marble Mountains; NB, North Bristol Mountains; NE, Newberry/Ord/Rodman Mountains; OD, Old Dad Peak/Marl/Kelso Mountains; OE, Indian Spring/Club Peak; PI, Piute Range; PR, Providence Range; WO, Wood Mountains

Assignment tests (STRUCTURE) and tests for migrants showed cross-interstate movements in TP2 but not TP1. For STRUCTURE analyses, we selected k = 5 (Figure S2). Results for each time point analysed separately were concordant with the combined analyses (not

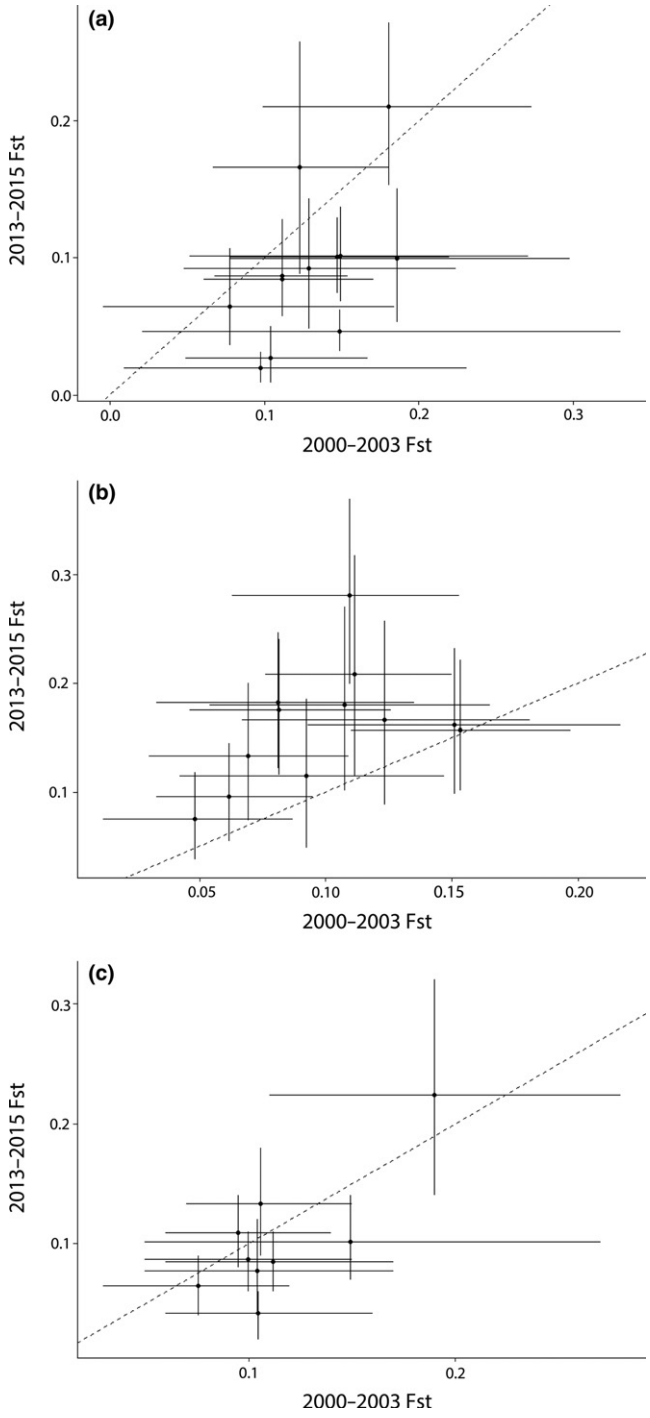

FIGURE 4 Population pairwise F_{ST} estimates among 13 populations of desert bighorn sheep in the Mojave Desert of California, based on 15 microsatellite markers, contrasted across two sampling periods (2000–2003 and 2013–2015), with 95% confidence intervals estimated by bootstrapping across loci. The dashed line marks identity between the time points, thus separating population comparisons for which genetic structure has decreased (below) and those that have increased (above). Values are shown for population pairs including the North Bristol Mountains (a), Piute Range (b) and populations near to but separated by Interstate 40 (c), including the Marble-Granite comparison (starred) where gene flow across the interstate was detected in Time Point (TP) 2 but not TP1. Although confidence intervals are large due to small sample size at TP1, most point estimates for the North Bristol Mountains (a) fall below the line, suggesting a general increase in genetic similarity with other populations in the study area, whereas all those for the Piute Range (b) all fall above the line, suggesting a general decrease in genetic similarity. Cross-interstate comparisons (c) showed little change except the Marble-Granite comparison

shown). At $k = 5$, all clusters had multiple individuals assigned at high confidence (q_{max} = 0.969–0.996). Both population average (Figure 1) and individual assignments (Figure S3) showed no evidence of cross-interstate movements among any population pairs at TP1 (e.g., North-South Bristol, Marble-Granite, Marble-North Bristol, Clipper-Providence). At TP2, however, the Granite Mountain populations showed clear contribution of individuals from populations south of Interstate 40 (Marble, Clipper or South Bristol Mountains, Figure 1), with five individuals assigned across the interstate at $q > 0.4$ (Figure 1). GENECLASS2 analyses identified one of those individuals as a migrant from the Marble Mountains (Table 3). One animal in the North Bristol Mountains showed ~40% assignment to the cluster south of the Interstate (Figure S3) and was identified as a migrant from the Marble Mountains (Table 3), but no evidence of potential direct movements between North and South Bristol Mountains was seen. Finally, in both analyses, no animals sampled south of the Interstate 40 appeared to be of northern origin (Figure 1, Table 3; Figure S3).

Other changes in connectivity were detected by assignment tests, tests for migrants and F_{ST} estimates. The North Bristol population was much more connected at TP2 (Figures 2 and 4). At TP1, this population was linked most closely to Old Dad Peak, likely due to remnant males from a prior translocation attempt (see Section 2.1). Since 2004, however, additional artificial water sources were developed, and a reproducing population was observed by 2009 (Abella et al., 2011). By TP2, genetic structure between North Bristol and nearby populations declined sharply (Figures 2 and 4), suggesting very frequent interpopulation movements were then occurring with the Cady Mountains and Granite Mountains, and to a lesser degree with Old Dad Peak (Figures 2 and 4; Figure S3, Tables S3, S4). This pattern was further supported by detection of migrants among those populations in TP2 (Table 3) and by individual assignments (Figure 1; Figure S3). The South Soda Mountains population, suspected to have been colonized from the Cady Mountains (J. Wehausen, personal communication, July 3, 2012), showed that link very clearly (Figure 1; Figure S3, Table S3, F_{ST} = 0.028).

Some changes were unsuspected prior to this analysis. At TP1, both Old Dad Peak and Indian Spring populations appeared completely isolated except from each other, although North Bristol animals showed significant Old Dad Peak heritage presumably due to the reintroduction attempt. Individual assignments (Figure 1; Figure S3) and detection of migrants (Table 3) showed clear evidence of new gene flow at TP2 to Old Dad Peak and Indian Spring from populations to the east (Providence, Wood, Hackberry and Piute cluster), and at least one individual at Indian Spring with Cady or

TABLE 2 Genetic distance (population pairwise F_{ST}) between desert bighorn sheep populations along Interstate 40, based on 15 microsatellite loci, from 2000–2003 and 2013–2015, with change in mean genetic distance (ΔF_{ST}) between time periods

Population pair	F_{ST} 2000–2003 (95% CI)	F_{ST} 2013–2015 (95% CI)	ΔF_{ST}	% change in 2000–2003 F_{ST}
CL-GR	0.08 (0.03–0.12)	0.07 (0.04–0.09)	−0.01	−14
CL-PR	0.10 (0.06–0.14)	0.11 (0.08–0.14)	0.01	15
KD-NE	0.19 (0.11–0.28)	0.22 (0.14–0.32)	0.03	18
KD-SB	0.11 (0.07–0.15)	0.13 (0.09–0.18)	0.03	26
MA-GR	**0.105 (0.062–0.156)**	**0.042 (0.020–0.064)**	**−0.063**	**−60**
MA-NB	0.11 (0.06–0.17)	0.09 (0.06–0.11)	−0.03	−24
MA-PR	0.10 (0.05–0.15)	0.09 (0.06–0.11)	−0.01	−13
SB-GR	0.10 (0.05–0.17)	0.08 (0.04–0.12)	−0.03	−26
SB-NB	0.15 (0.05–0.27)	0.10 (0.07–0.14)	−0.05	−32

Only comparisons across the highway are shown. The Marble (MA) and Granite (GR) Mountains pair (bolded) showed the most direct evidence for cross-interstate movements during 2013–2015.

South Soda Mountains heritage (Table 3; Figure S3). Beyond the changes detailed above, however, genetic distances and patterns of assignment to STRUCTURE clusters showed little change among many populations (Table S4; Figure S3).

3.3 | Genetic diversity

Before estimating genetic diversity, we removed locus BL4 because of evidence of positive selection at that locus (Appendix S1). For estimating allelic richness (A_r), we also removed locus OarFCB266 because it mostly failed in the small North Bristol sample at TP1. Genetic diversity changed little in most populations (Table S5). Genetic diversity in two populations apparently linked by new movements across Interstate 40 (Granite and Marble Mountains) did not change significantly: average expected heterozygosity (H_e) across 15 loci did not increase significantly in either population (pairwise Wilcoxon rank-sum tests; Granite Mountains, mean $H_{e\_TP1} = 0.66$, mean $H_{e\_TP2} = 0.69$, $S = -22.0$, $p = .11$; Marble Mountains, mean $H_{e\_TP1} = 0.66$, mean $H_{e\_TP2} = 0.67$, $S = -19.5$, $p = .14$). Using a minimum per-locus sample size of 15 in those two populations across time points, average A_r across 15 loci also did not change significantly between time points in the Granite Mountains (pairwise Wilcoxon rank-sum tests, $A_{r\_TP1} = 4.62$, $A_{r\_TP2} = 4.83$, $S = -20.5$, $p = .12$) or in the Marble Mountains ($A_{r\_TP1} = 4.20$, $A_{r\_TP2} = 4.26$, $S = -14.0$, $p = .24$). Considering only those two populations, 17 alleles in each time step were private to one or the other population (TP1: MA, $n = 4$ alleles, GR, $n = 13$; TP2: MA, $n = 5$, GR, $n = 12$), although the identity of the private alleles varied across time points in some cases.

4 | DISCUSSION

We observed significant localized changes in genetic structure, supporting our hypothesis that both structural and functional connectivity changed among populations of desert bighorn sheep in the central Mojave Desert of California after only two generations (~12 years). These changes appeared to be driven in part by colonization and population expansion into habitats apparently unoccupied or transiently occupied c. 2000–2003 (TP1), but also by apparent changes in willingness or ability of bighorn sheep to move across or under a fenced four-lane highway (Interstate 40) in at least one location (north end of Marble Mountains). In particular, between time points, we observed a twofold decrease in genetic distance between two populations in mountain ranges separated by that highway, detected via genotype recapture one bighorn sheep using both ranges and detected two individuals assigned as migrants across the highway. In contrast, in TP1, we saw no cross-interstate assignment of individuals or migrants. Thus, we conclude that between TP1 and TP2, bighorn sheep began crossing Interstate 40 in at least one location. We know of no change in structural barriers or decrease in traffic over this time. Other populations separated by that highway still showed no clear evidence of increased gene flow or cross-assignment of individuals since 2000–2003 (Tables 2 and 3; Figure S3), suggesting that the fenced highway typically still acts as a barrier.

Although population genetic approaches often are not precise at detecting occasional or short-term interpopulation movements (Lowe & Allendorf, 2010), we suggest that the gene flow across the interstate highway between the Marble and Granite and possibly the Marble and North Bristol Mountains detected at TP2 is a new pattern of movement, and was not simply "missed" at TP1. In addition to our analyses, a summary of radiotelemetry data collected in the region over more than a decade prior to TP1 likewise showed no confirmed crossings (Epps et al., 2007). Nor do we ascribe the change in genetic structure to a time-lagged response to movements before TP1 (Epps & Keyghobadi, 2015), because individual assignments and migrant tests among these genetically distinct populations would offer immediate detection of new connections. Sample sizes were larger in some populations in TP2 (Table 1), likely increasing chances of detecting migrants by assignment tests or genetic recapture. However, the analysis of all samples from both time points in STRUCTURE would be less influenced by sample size differences. Our simulations of power to detect change in F_{ST} over different sample sizes also suggest reasonable power to resolve differences among most populations (Figure S1), particularly in the Marble-Granite

TABLE 3 First-generation (i.e., F0, Paetkau et al., 2004) migrants detected among desert bighorn sheep during 2000–2003 and 2013–2015, using GENECLASS2 and 15 microsatellite loci

Time period	Population where migrant detected	Inferred source population	Instances	*p*
2000–2003	Clipper Mtns (CL)	Marble Mtns (MA)	2	.0081, .0031
	Granite Mtns (GR)	Old Dad Peak (OD)	1	.0025
	Granite Mtns (GR)	Providence Mtns (PR)	1	.0095
	Granite Mtns (GR)[a]	Old Dad Peak (OD)	1	.0023
	Cady Mtns (KD)[a]	Old Dad Peak (OD)	1	.0001
	Marble Mtns (MA)	South Bristol Mtns (SB)	1	.0099
	Old Dad Peak (OD)	Indian Spring (OE)	2	.0041, .0025
	Indian Spring (OE)	Old Dad Peak (OD)	1	.0077
	Piute Range (PI)	Wood Mtns (WO)	1	.0019
	Providence Mtns (PR)	Piute Range (PI)	1	.0001
2013–2015	Clipper Mtns (CL)	Marble Mtns (MA)	1	.0038
	Granite Mtns (GR)	**Marble Mtns (MA)**	**1**	**.0098**
	Cady Mtns (KD)	Old Dad Peak (OD)	1	.0012
	North Bristol Mtns (NB)	Granite Mtns (GR)	2	.0010, .0038
	North Bristol Mtns (NB)	**Marble Mtns (MA)**	**1**	**.0083**
	North Bristol Mtns (NB)	Old Dad Peak (OD)	1	.0049
	Newberry Mtns (NE)[b]	Indian Spring (OE)	1	.0002
	Old Dad Peak (OD)	Indian Spring (OE)	1	.0094
	Old Dad Peak (OD)	Wood Mtns (WO)/Piute Range (PI)	1	<.0001
	Indian Spring (OE)	Old Dad Peak (OD)	1	.0087
	Indian Spring (OE)	Piute Range (PI)	1	.0099
	Indian Spring (OE)	South Soda Mtns (SS)	1	.0001
	Piute Range (PI)	Wood Mtns (WO)	1	.0008
	Providence Mtns (PR)	Hackberry Mtns (HA)	1	.0283
	Providence Mtns (PR)	South Soda Mtns (SS)/Cady Mtns (KD)	1	<.0001
	South Bristol Mtns (SB)	Marble Mtns (MA)	1	.0007
	South Soda Mtns (SS)	Granite Mtns (GR)	1	.003

We used a significance threshold of $p < .01$ to identify potential migrants. Near-ties in inferred source population (i.e., likelihood estimates differing by <1) are noted by listing >1 population. Migrants from populations across Interstate 40 are noted in bold.

[a]These assignments may be most parsimoniously explained as resulting from Old Dad Peak individuals that were translocated to the North Bristol Range in 1992 (Wild Sheep Working Group 2015) and subsequently migrated, as is common after a translocation, rather than natural movements from Old Dad Peak.

[b]This assignment results from recent gene flow between Newberry Mountains and the Sheephole Mountains (C. Epps, unpublished data), also south of Interstate 40, which received a transplant of Old Dad Peak individuals in 1984 (Wild Sheep Working Group 2015). Indian Spring is sometimes considered a subpopulation of Old Dad Peak due to movement by collared animals among those areas (Bleich, Whiting, Kie, & Bowyer, 2016).

comparison given robust sample sizes there. Thus, all lines of evidence consistently pointed to a change in movement patterns in this location. Fragmentation by roads and other linear anthropogenic features has been posited as a leading cause of habitat fragmentation and direct mortality for wildlife worldwide (Trombulak & Frissell, 2000). Our study in no way contradicts that conclusion, but does offer evidence that species may interact with such barriers in different ways over time.

This apparent change in willingness for crossing an interstate highway—albeit apparently only in one location—highlights the need for caution in extending inferences generated from a single estimate of functional connectivity forward in time (Farrington & Petren, 2011). Animal movement behaviours may be more plastic than we often recognize, particularly when such behaviours may be learned. Much attention has been given to the need to consider and estimate functional connectivity (Milanesi, Holderegger, Bollmann, Gugerli, &

Zellweger, 2017; Turgeon, Robillard, Gregoire, Duclos, & Kramer, 2010), particularly from empirical data, and individual variation in functional connectivity has likewise been recognized (Belisle, 2005). Even a single empirical estimate of functional connectivity can pose a significant challenge. Yet, the proliferation of studies of landscape ecology (Urban, Oneill, & Shugart, 1987) or landscape genetics (Manel, Schwartz, Luikart, & Taberlet, 2003) offers opportunities to revisit such estimates across a variety of systems, potentially shedding light on when repeated studies may be most warranted.

The largest shifts in genetic structure within the same populations (Figure 3) as well as between populations (Figures 2 and 4) appear to have been caused by establishment and subsequent expansion of new populations, which may be considered changes in structural connectivity. The small sizes of these populations (Table 1) make them particularly subject to rapid changes in genetic structure, as demonstrated by strong genetic structure at TP1 that apparently resulted from construction of barriers only ~7 generations before (Epps et al., 2005). An apparent recolonization of the North Bristol Mountains demonstrated how population restoration in a central location in a network can sharply increase gene flow and forge new links among populations over even a short period of time (Figure 2). Based on the genetic characteristics of this metapopulation at TP2 (Figure 1; Figure S3), we conclude the reestablishment of the North Bristol population was likely driven by expansion of the bighorn population in the Cady Mountains (Abella et al., 2011), and perhaps influenced by the installation of artificial water sources installed in the North Bristol Mountains around the time of the TP1 study. Other changes imply hitherto-unsuspected shifts in distribution and movements of bighorn sheep among mountain ranges, in some cases likely driven by dynamics outside of the study area (e.g., Piute Range, Figures 1, 3, 4).

Because no animal sampled south of Interstate 40 during 2013–2015 was assigned to populations north of the highway (Table 3; Figure S3), cross-highway gene flow seems largely driven by bighorn sheep originating south of Interstate 40 (likely, the Marble Mountains). Dispersal in bighorn sheep may best be described as facultative adult dispersal, as adults of both sexes and a variety of ages occasionally make long-distance or exploratory movements, but the behaviour is highly variable among individuals (O'Brien, O'Brien, McCarthy, & Carpenter, 2014). The gregarious nature of this species may mean that once a single individual has determined a new movement route, others will follow. Population expansion may also influence willingness of individuals to undertake potentially risky movements, as observed in other large herbivores with density-dependent dispersal (Labonte, Ouellet, Courtois, & Belisle, 1998), although larger populations may just produce increased numbers of dispersers. Populations have increased in the Marble Mountains and particularly the Cady Mountains in recent decades (Abella et al., 2011; Torres et al., 1994). Both populations served as source populations for natural recolonizations (this study, see also Epps et al., 2010). Thus, for this and other species exhibiting facultative adult dispersal, clarifying which individual- and population-level characteristics are associated with increased dispersal rates or numbers could improve our understanding of interpopulation movement or the potential for it to occur and thereby facilitate management of spatially complex systems.

Despite the dramatic increases in gene flow among several pairs of populations, only relatively small changes in genetic diversity have occurred in the affected populations over the 12-year interim between sampling, even where movement has now been established across man-made barriers present for >50 years. Genetic diversity is predicted to attain equilibrium more slowly than genetic structure after a change in migration rates (Epps & Keyghobadi, 2015); thus, we expect that genetic diversity will increase in future generations among those populations linked by new connections (e.g., Marble and Granite Mountains, Figure 1), unless influenced by other events such as population bottlenecks. The sharpest increase in genetic diversity occurred in the North Bristol Mountains, where a newly established or expanded population created a central connection among three or four populations (Figures 1 and 4), again pointing to the importance of connectivity and immigration in maintaining genetic diversity of metapopulations (Farrington & Petren, 2011). Many connections throughout the study area showed little evidence of change, however, so we do not expect a general trend of increased or decreased diversity across the study area (Table S5, Figure S3).

Our findings also shed light on the recent discovery of respiratory disease throughout much of the study system. The pattern of high gene flow links (i.e., $F_{ST} \leq 0.05$, Epps et al., 2010) observed among populations in 2013–2015 (Figure 2) corresponds exactly with the distribution of the single strain of *M. ovi* detected in the study area in 2013–2015 (CDFW, unpublished data). We documented a substantial decrease in the isolation of the Old Dad Peak/Kelso/Marl Mountains and Indian Spring populations, which we propose could in part explain the different response to respiratory disease observed there in 2013. In 2000–2003, these populations were strongly genetically distinct from other nearby populations (Figure 1; Table 3). By 2013–2015, interbreeding had occurred with the Providence-Wood-Hackberry-Piute chain of populations to the east and the North Bristol and connected ranges to the south (Figures 1 and 2, Tables 3; Figure S3, Table S4). During the recent outbreak of respiratory disease (2013-present), Old Dad Peak was the only population known to have experienced an all-ages die-off (CDFW, unpublished data). One hypothesis for this variable pattern of mortality, supported by serology tests (CDFW, unpublished data), is that other populations in the area had previously experienced *M. ovi* outbreaks, but Old Dad Peak had not because of its isolation.

Systematic genetic sampling in this metapopulation of large, long-lived mammals at time points separated by only two generations (12 years) revealed a hitherto-unsuspected degree of dynamism in genetic structure and, apparently, movement behaviour. We interpret these changes as resulting from population expansions, recolonizations and a change in functional connectivity, that is, willingness to cross an anthropogenic barrier. Our findings further support the use of population genetics as a way to obtain a high-resolution, systematic picture of metapopulation structure (Lamy, Pointier, Jarne, &

David, 2012), particularly when populations are small, but also make it clear that such characterizations may need revisiting. Moreover, we conclude that movement models based on any single estimate of movement patterns, whether genetic-based (Cushman et al., 2006; Epps et al., 2007) or telemetry-based (Chetkiewicz & Boyce, 2009), should be reviewed periodically. As future opportunities occur for recharacterizing animal movements in well-studied systems, we predict that systems with frequent population turnover, strong shifts in population density, or with long-lived species capable of learning behaviours from other individuals would be most likely to experience strong shifts in movement patterns.

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DATA ACCESSIBILITY

Sampling locations and 16-locus microsatellite genotypes of all individuals for both time periods are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.mp71t50.

AUTHOR CONTRIBUTIONS

C.W.E. designed the research; C.W.E. and R.S.C. conducted the field work; R.S.C., B.N. and C.W.E. conducted the laboratory work; C.W.E. analysed the data; C.W.E., R.S.C., B.N. wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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