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Rapid genetic restoration of a keystone species exhibiting delayed demographic response

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Abstract

Genetic founder effects are often expected when animals colonize restored habitat in fragmented landscapes, but empirical data on genetic responses to restoration are limited. We examined the genetic response of banner-tailed kangaroo rats (*Dipodomys spectabilis*) to landscape-scale grassland restoration in the Chihuahuan Desert of New Mexico, USA. *Dipodomys spectabilis* is a grassland specialist and keystone species. At sites treated with herbicide to remove shrubs, colonization by *D. spectabilis* is slow and populations persist at low density for ≥10 years (≥6 generations). Persistence at low density and low gene flow may cause strong founder effects. We compared genetic structure of *D. spectabilis* populations between treated sites and remnant grasslands, and we examined how the genetic response to restoration depended on treatment age, area, and connectivity to source populations. Allelic richness and heterozygosity were similar between treated sites and remnant grasslands. Allelic richness at treated sites was greatest early in the restoration trajectory, and genetic divergence did not differ between recently colonized and established populations. These results indicated that founder effects during colonization of treated sites were weak or absent. Moreover, our results suggested founder effects were not mitigated by treatment area or connectivity. Dispersal is negatively density-dependent in *D. spectabilis*, and we hypothesize that high gene flow may occur early in the restoration trajectory when density is low. Our study shows genetic diversity can be recovered more rapidly than demographic components of populations after habitat restoration and that founder effects are not inevitable for animals colonizing restored habitat in fragmented landscapes.

Keywords: conservation genetics, density dependence, *Dipodomys spectabilis*, dispersal, ecological restoration, founder effects

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Introduction

Because population viability is sensitive to genetic factors (Frankham 2005), restoration of genetic diversity has become a critical component of conservation efforts. Over short timescales, genetic drift and inbreeding reduce genetic diversity in small populations, and inbreeding depression increases the probability of local extinction (Saccheri *et al.* 1998; Westemeier *et al.* 1998; Reed *et al.* 2007). Over long timescales, lack of genetic diversity increases extinction risk by constraining adaptive evolutionary responses to environmental change (Reed *et al.* 2003; Frankham & Kingsolver 2004; Frankham 2005). These factors are often incorporated into genetic restoration plans involving the intentional movement of individuals (i.e. translocations; Weeks *et al.* 2011), but less is known about genetic processes when species passively colonize restored habitat (Brudvig 2011), especially animals (Mijangos *et al.* 2015).

According to metapopulation genetic theory, genetic diversity during colonization is shaped by size of the

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founding population and diversity of source populations from which founders originate (Slatkin 1977; Wade & McCauley 1988; Whitlock & McCauley 1990; Pannell & Charlesworth 2000). Limited genetic diversity and high genetic divergence occur when founding populations are small and colonists originate from few source populations (e.g. Whitlock & McCauley 1990; Giles & Goudet 1997; Cosentino *et al.* 2012). Strong founder effects are often expected for species colonizing restored habitat because those species typically occur in landscapes that have a recent history of habitat fragmentation (Vandepitte *et al.* 2012).

Founder effects at restoration sites may be mitigated by extrinsic, geographical factors that shape dispersal. For example, spatial connectivity of restoration sites to multiple source populations can facilitate rapid genetic restoration (Helsen *et al.* 2013, 2015). Additionally, maximizing the size of restoration sites could reduce founder effects if immigration is positively related to habitat area (i.e. target effect; Cosentino *et al.* 2012). However, dispersal is not a 'neutral' trait that depends only on geography (Lowe & McPeek 2014). Variation in dispersal strategies among species may play an important role in determining the likelihood of founder effects. For example, among small mammals with fluctuating population density, theory predicts that population genetic structure depends on the type of density-dependent dispersal (Charnov & Finerty 1980; Lambin & Krebs 1991). When immigration is positively related to density, genetic diversity should be reduced at low density due to strong genetic drift and low gene flow (e.g. Berthier *et al.* 2006; Gauffre *et al.* 2014). When immigration is negatively related to density, genetic diversity should be maintained at low density because gene flow compensates for strong genetic drift (e.g. Ehrich *et al.* 2009; Pilot *et al.* 2010). When colonizing restored habitat, species with negative density-dependent immigration may have weak founder effects due to a large influx of immigrants at low density, particularly if immigrants arrive from multiple source populations.

We examined the genetic response of an ecologically important vertebrate to landscape-scale habitat restoration. Shrub encroachment is a global problem in arid and semi-arid ecosystems (Archer 2010). In the Chihuahuan Desert of the southwestern United States, shrublands dominated by creosotebush (*Larrea tridentata*) and honey mesquite (*Prosopis glandulosa*) have replaced grasslands because of livestock overgrazing, drought, lack of fire, and cross-scale feedbacks among grass loss, soil erosion, and shrub dominance (Peters *et al.* 2006; Archer 2010). In our study area in southern New Mexico, shrub encroachment has occurred in >50% of grassland and savannah habitat (Gibbens *et al.* 2005; Yanoff *et al.* 2008). The banner-tailed kangaroo rat (*Dipodomys spectabilis*) is a grassland specialist that has declined in response to woody encroachment (Waser & Ayers 2003). Populations are extirpated when shrub cover exceeds 15–20% (Krogh *et al.* 2002; Cosentino *et al.* 2014), which is typical of shrublands in the region (Coffman *et al.* 2014). Impacts of shrub invasion are alarming because – as a keystone species – *D. spectabilis* strongly shapes the structure and function of desert grasslands by altering vegetation via selective granivory and building large mounds (2–5 m wide) that create refuge for other species and affect species abundances and community composition (e.g. Brown & Heske 1990; Hawkins & Nicoletto 1992; Guo 1996; Schooley & Wiens 2001; Davidson & Lightfoot 2006; Cosentino *et al.* 2013; McAllister *et al.* 2014). Maintaining genetic diversity will be essential for *D. spectabilis* to adapt to environmental change and sustain keystone functions.

In the 1980s, the Bureau of Land Management (BLM) began using herbicides and livestock grazing deferment over large areas of southern New Mexico to remove shrubs and restore grasslands. These efforts were expanded in 2005 as part of the Restore New Mexico program. Over 1 000 000 ha have been treated statewide since 2005, and >1 500 000 ha are targeted for future treatments (http://www.blm.gov/nm/st/en/prog/restore_new_mexico.htlml). Treatments to control creosotebush have reduced shrub cover, increased perennial grass cover, and increased the abundance of animal species that are grassland specialists (Cosentino *et al.* 2013; Coffman *et al.* 2014; McAllister *et al.* 2014).

Dipodomys spectabilis populations occur as clusters of mounds in grassland habitat (Schooley & Wiens 2001; Skvarla *et al.* 2004; Sanderlin *et al.* 2012). Herbicide treatment targeting shrubs has increased the abundance of *D. spectabilis* populations, but this positive response has a time lag of ≥10 years (Cosentino *et al.* 2014) despite a short generation time (1.7 years; Swanson 2001). Most *D. spectabilis* individuals disperse short distances (<100 m; Skvarla *et al.* 2004; Waser *et al.* 2006), and connectivity to source populations facilitates the positive demographic response to treatments (Cosentino *et al.* 2014). Source populations in the region are assumed to primarily occur in other areas treated with herbicide because remnant grasslands are rare, and *D. spectabilis* density is almost an order of magnitude greater in treated areas than in untreated shrublands (Cosentino *et al.* 2014).

Our primary objective was to evaluate whether strong founder effects occur during colonization of restored habitat. Because *D. spectabilis* has limited dispersal ability and populations persist at low density early in the restoration trajectory, genetic drift and inbreeding may constrain genetic diversity initially. These forces could be offset by gene flow and increasing population size as

time since treatment increases. Three predictions would support founder effects at restoration sites: (i) genetic diversity should be positively related to time since treatment, (ii) genetic divergence among recently colonized populations should be greater than genetic divergence among older populations (e.g. Whitlock 1992; Giles & Goudet 1997; Cosentino *et al.* 2012) and (iii) mean genetic diversity should be lower at treated sites than at remnant grasslands due to limited genetic diversity at recently colonized sites.

Alternatively, founder effects may be absent due to high gene flow. Studies of *D. spectabilis* indicate dispersal probability, dispersal distances and survival of dispersers are greater at low density than at high density (Jones 1988; Jones *et al.* 1988; Waser *et al.* 2006). Furthermore, Sanderlin *et al.* (2012) showed that the contribution of immigration to growth of local populations in a metapopulation was greater at low metapopulation density than at high metapopulation density. Dispersal is risky at high density because vacant mounds are limited and constructing a new mound can take >1 year and is energetically costly (Best 1972; Jones 1984, 1988). Negative density-dependent immigration could cause high gene flow early in the restoration trajectory, counterbalancing genetic drift.

Because geography can affect the strength of gene flow, we also assessed whether the genetic response of *D. spectabilis* to treatments was related to the size and spatial configuration of treated areas. Density of *D. spectabilis* was related positively to connectivity to source populations but unrelated to treatment area (Cosentino *et al.* 2014). Assuming gene flow increases with spatial connectivity, we predicted that genetic diversity at treated sites would be positively related to connectivity to source populations.

Materials and methods

Study sites and tissue collection

We sampled 20 sites in a 1 537 433 ha area centred near Hatch, New Mexico in the northern Chihuahuan Desert (Fig. 1). Median pairwise distance between sites was 65 km (range = 2–133 km). A single application of the herbicide tebuthiuron was used to treat 18 sites to target creosotebush from 1982 to 2006 (5–29 years before our study), and median area of treatments was 699 ha (range = 193–1840 ha). Two sites were located in unencroached, remnant grasslands never treated with herbicide, and we used these sites as references to compare genetic diversity between restored and remnant populations. We attempted to obtain a more balanced sample of treated and remnant sites, but populations in remnant grasslands are extremely rare in the region.

We sampled an average of 21 adults at each site (range = 13–31; Table 1). Adults were captured by setting 2–3 Sherman live traps at mounds (Jones 1984) from September–November 2011 (12 sites) and March–April 2012 (8 sites). The median minimum convex polygon including all sampled adults at each site was 28 ha (range = 11–153 ha). We distinguished adults from juveniles by reproductive maturity (descended testes or nipples ≥1 mm long; Waser *et al.* 2006) and size (≥100 g). Tissue samples were collected by removing a 1 × 4-mm section of ear tissue from each individual. Tissue samples were placed in 95% ethanol and stored in a −20 °C freezer before DNA extraction.

Microsatellite amplification and scoring

DNA was extracted from tissue samples with a Qiagen DNeasy Blood and Tissue kit (Qiagen, Inc., Valencia, CA, USA). We genotyped individuals at seven microsatellite loci developed for *Dipodomys spectabilis* (DS01, DS03, DS46, DS98, DS107, DS109 and DS163; Davis *et al.* 2000; Waser *et al.* 2006). PCRs consisted of 10 μL volumes containing 1X buffer (Promega) 2.0–2.5 mM $MgCl_2$ (Promega), 0.2 mM dNTPs (Promega), 0.07–0.30 μM forward and reverse primers, and 1.25–1.50 units *Taq* DNA polymerase (Promega). PCR profiles were previously described (Davis *et al.* 2000; Waser *et al.* 2006). Forward primers were labelled with fluorescent dyes for genotyping, and PCR products were visualized on an ABI Prism 3730xl Analyzer (Applied Biosystems, Foster City, CA, USA). Alleles were scored manually with GENEMAPPER 3.7 (Applied Biosystems) and binned with TANDEM v1.09 (Matschiner & Salzburger 2009). MICROCHECKER 2.2.3 (van Oosterhout *et al.* 2004) indicated that genotyping errors and null alleles were not present.

Genetic diversity and population structure

We used exact tests in GENEPOP 4.3 to test for departures from Hardy–Weinberg equilibrium at each locus and gametic equilibrium at each pair of loci (Markov chain method, 10 000 dememorization steps, 1000 batches, 10 000 iterations per batch; Raymond & Rousset 1995). We used the package HIERFSTAT (Goudet & Jombart 2015) in R 3.1.2 (R Core Team 2014) to calculate three metrics of genetic diversity for each site-locus combination: expected heterozygosity (H_E), observed heterozygosity (H_O), and allelic richness (A_R) corrected for sample size. We quantified bias-corrected percentile bootstrap confidence intervals for H_E, H_O, and A_R with 10 000 bootstrap replications (BCa method; Efron 1987). The BCa method is more accurate than the standard percentile method when the bootstrap distribution is

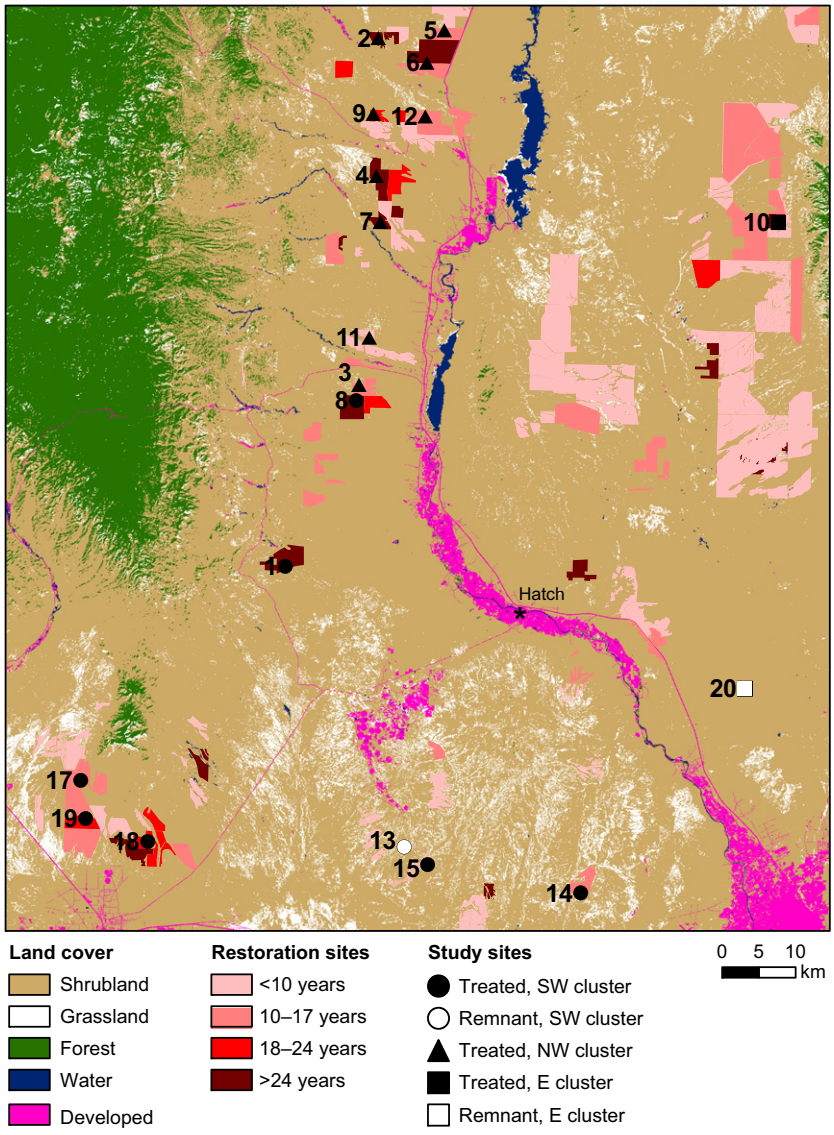

Fig. 1 Map of land cover, sites treated with herbicide to restore grassland and study sites for *Dipodomys spectabilis* in southern New Mexico, USA. Areas treated with herbicide are represented by a colour gradation corresponding to treatment age. Symbols (circles, triangles and squares) represent study areas, and the adjacent number represents the site number. Symbol fill represents treatment, and symbol shape represents genetic cluster inferred by a Bayesian clustering analysis.

skewed or asymmetric about the parameter estimate (Efron 1987), which we observed frequently for bootstrap distributions of H_E, H_O, and A_R. FSTAT 2.9.3 was used to estimate global F_{IS} and F_{ST} (Weir & Cockerham 1984) by jackknifing across loci, pairwise F_{ST} between all site pairs and F_{IS} for each site (Goudet 1995). Permutation tests were used to determine the significance of pairwise F_{ST} values (3800 permutations) and F_{IS} for each site (2800 permutations; Goudet 1995). In cases where multiple statistical tests were performed, we used a sequential Bonferonni correction to maintain a family-wise error rate of $\alpha = 0.05$ (Rice 1989).

We characterized population structure in two ways. First, we used a Bayesian clustering method implemented in STRUCTURE 2.3.4 to examine the genetic structure of sites without defining populations *a priori* (Pritchard *et al.* 2000). The goal of this analysis was to identify broad-scale population structure that may confound analyses of genetic diversity and divergence at the site-level. We used the correlated allele frequency model (Falush *et al.* 2003) and the admixture model in which a proportion of each individual's genome is assigned to one or more of K populations. We also used a location prior (Hubisz *et al.* 2009), which specifies the site at which each individual was sampled and can improve detection of genetic structure when overall genetic divergence is weak. We examined values of K from 1 to 20. We ran 15 simulations at each value of K, and each run consisted of 500 000 iterations following a 100 000-iteration burn-in period. We determined support for K by examining the second order rate of change of mean log probability for sequential values of K (ΔK method; Evanno *et al.* 2005). STRUCTURE HARVESTER was used to quantify ΔK (Earl & vonHoldt 2012). The STRUC-

Table 1 Site number, number of adults sampled (N), treatment (treated with herbicide vs. remnant grassland), age (years since treatment with herbicide), area of treated sites, connectivity to source populations and metrics of genetic structure for *Dipodomys spectabilis* in the Chihuahuan Desert of southern New Mexico, USA. Metrics of genetic structure include expected (H_E) and observed heterozygosity (H_O), inbreeding coefficient (F_{IS}) and allelic richness corrected for sample size (A_R) averaged across loci. F_{IS} values were not significantly different from 0 after a sequential Bonferonni correction. Locus-specific estimates and 95% confidence intervals of H_O, H_E, and A_R can be found in Tables S1–S3 (Supporting information).

Site	N	Type	Age (year)	Area (ha)	Connect	H_E	H_O	F_{IS}	A_R
1	28	Treated	29	1294	0.00	0.69	0.68	0.02	5.95
2	22	Treated	23	295	2.05	0.70	0.71	−0.01	6.24
3	24	Treated	12	610	3.16	0.69	0.67	0.03	6.02
4	20	Treated	23	1004	1.46	0.68	0.69	−0.02	6.22
5	22	Treated	11	665	4.21	0.69	0.62	0.10	6.37
6	19	Treated	24	1840	7.38	0.65	0.62	0.05	5.71
7	19	Treated	25	408	3.41	0.71	0.74	−0.04	5.87
8	25	Treated	24	987	4.44	0.71	0.70	0.02	6.01
9	15	Treated	16	294	1.07	0.68	0.69	−0.03	6.72
10	13	Treated	23	449	5.28	0.79	0.78	0.01	7.57
11	20	Treated	8	265	2.39	0.72	0.70	0.03	6.31
12	17	Treated	11	1394	2.12	0.68	0.72	−0.06	6.17
13	24	Remnant	NA	NA	NA	0.69	0.70	−0.01	6.38
14	31	Treated	11	826	0.00	0.73	0.74	−0.02	6.77
15	15	Treated	5	682	0.00	0.71	0.67	0.06	6.75
16	19	Treated	7	193	1.01	0.71	0.73	−0.03	6.49
17	22	Treated	12	878	3.64	0.70	0.70	0.00	6.10
18	21	Treated	18	727	5.27	0.66	0.65	0.03	6.33
19	24	Treated	18	715	5.71	0.67	0.67	0.00	6.41
20	19	Remnant	NA	NA	NA	0.79	0.78	0.01	6.86

TURE analysis clearly identified two clusters separated by the Rio Grande, and we further examined the cluster west of the Rio Grande for substructure because it included 18 of 20 sites. We examined values of K from 1 to 18 in the west cluster using the same methods for the analysis of all sites.

Second, we used a Mantel test to assess isolation by distance (IBD) by examining the relationship between pairwise genetic distance (F_{ST} / (1 – F_{ST})) and the natural logarithm of geographical distance between all sites (Rousset 1997). Because the STRUCTURE analysis revealed two genetic clusters separated by the Rio Grande, we also used a separate Mantel test to examine IBD for the 18 sites west of the Rio Grande. Mantel tests were conducted with the ISOLATION BY DISTANCE WEB SERVICE 3.23 (Jensen *et al.* 2005) with 10 000 randomizations.

Genetic response to restoration

First, we examined whether genetic structure at treated sites was related to treatment age (i.e. years since herbicide treatment), area of treated sites and connectivity of treated sites to source populations. Source populations were defined as areas previously treated with herbicide because of low abundance of *D. spectabilis* in shrublands and the rarity of remnant grasslands (Cosentino *et al.* 2014). We quantified connectivity using an incidence function-based metric that incorporated proximity, area and age of potential source populations (Hanski 1994; Moilanen & Nieminen 2002; Cosentino *et al.* 2014). Connectivity (C_i) of each treated site was quantified as follows:

$$C_i = \sum_{j \neq i} \exp(-d_{ij}) A_j'^{b},$$

where d_{ij} is the distance between treated site i and source population j, A_j' is the effective area of source population j, and b is a parameter scaling the association between abundance and effective area of source populations. We included source populations in the calculation of connectivity if they were within 3 km of treated areas, which is consistent with the maximum dispersal distance of *D. spectabilis* (Skvarla *et al.* 2004). Effective area (Hanski 1994) was the area of source populations weighted by treatment age because density of *D. spectabilis* is positively related to treatment age (Cosentino *et al.* 2014). Effective area of source population j was calculated as Q_jA_j/Q^*, where Q_j was the treatment age of source j, Q^* was the maximum treatment age of any potential source, and Aj was the area of source j. We set b to 0.5 because the relationship

between emigration and effective area is not likely to be linear (Moilanen & Nieminen 2002). Prugh (2009) showed that the effect of estimated connectivity on model parameters is not sensitive to changes in *b*.

We used linear mixed models to examine how genetic diversity at sites (A_R, H_E) was related to treatment age, area and connectivity. We modelled variation in H_E and not H_O because there was no evidence for deviations from Hardy–Weinberg equilibrium and precision of estimates was greater for H_E than for H_O (Tables S1–S2, Supporting information). We used a Gaussian error distribution and identity link function for A_R and H_E. All explanatory variables were standardized. Genetic locus was specified as a random effect with a random intercept model to account for variation in A_R and H_E among loci (Tamaki *et al.* 2008). We excluded the only treated site east of the Rio Grande (Site 10; Fig. 1) from this analysis because of strong genetic divergence across the Rio Grande identified by the clustering analysis. We considered specifying genetic cluster as a random effect to account for substructure among sites. However, there was only one treated site in the cluster east of the Rio Grande, and exploratory analyses indicated the variance estimate for a random effect of genetic clusters west of the Rio Grande was zero.

For each response variable, we compared the fit of 8 models: (i) one model with an intercept only, (ii) three models with a single explanatory variable and (iii) four models with additive effects of explanatory variables. None of the explanatory variables were strongly correlated (all $r < 0.38$). Model support was examined using Akaike's information criterion (AIC), and models with ΔAIC ≤ 2 were considered to have competitive support (Burnham & Anderson 2002). All models were fit in R 3.1.2 (R Core Team 2014) with the LME4 package (Bates *et al.* 2014).

Second, we tested whether genetic divergence (F_{ST}) differed between recently colonized populations and established populations. Comparing genetic structure between recent and established populations has been widely used to infer founder effects (e.g. Whitlock 1992; McCauley *et al.* 1995; Giles & Goudet 1997), including in the restoration literature (e.g. Vandepitte *et al.* 2012; Neuwald & Templeton 2013; Helsen *et al.* 2015). Recently colonized populations were defined as sites treated for shrub removal within 5–16 years ($\leq$10 generations; $n = 9$; Table 1), and established populations were defined as sites treated >16 years earlier ($n = 8$; Table 1). We excluded the only treated site east of the Rio Grande from this analysis (Site 10; Fig. 1). Pairwise geographical distances were similar between recently colonized (mean = 69.0 km, SD = 38.6 km) and established populations (56.1 km, SD = 35.7 km). We used FSTAT 2.9.3 to compare F_{ST} between recently colonized and established populations with a permutation test (10 000 permutations; Goudet 1995).

Third, we examined whether grassland restoration efforts lead to recovery of genetic diversity of *D. spectabilis* by comparing genetic diversity between treated and remnant sites. We excluded the single treated site and single remnant site east of the Rio Grande from this analysis due to strong divergence across the Rio Grande (Sites 10 and 20; Fig. 1). We used a one-sample *t*-test to examine if genetic diversity (A_R, H_E) at treated sites west of the Rio Grande was consistent with the values of genetic diversity estimated at the single remnant site west of the Rio Grande (Site 13; Table 1). A_R and H_E were averaged across loci for this analysis. Power analyses indicated that power to detect $\geq$5% differences in genetic diversity from the remnant site was $\geq$0.90.

Results

Genetic diversity and population structure

There was no evidence of deviation from Hardy–Weinberg proportions or gametic disequilibrium after sequential Bonferonni corrections. Mean observed heterozygosity was 0.70 (range = 0.62–0.78; Tables 1 and S1, Supporting information), mean expected heterozygosity was 0.70 (range = 0.65–0.79; Tables 1 and S2, Supporting information), and mean allelic richness was 6.36 (range = 5.71–7.57; Tables 1 and S3, Supporting information). There was no evidence for a deficit or excess of heterozygotes within sites (mean $F_{IS} = 0.007$, SE = 0.01; Table 1).

Overall F_{ST} among all sites was 0.049 (SE = 0.007), and pairwise F_{ST} ranged from 0 to 0.19 (Table S4, Supporting information). There was a significant pattern of IBD among all sites (Fig. 2; $r = 0.31$, $P < 0.0001$). The STRUCTURE analysis showed support for $K = 2$ clusters based on the ΔK method (Fig. 3, Table S5, Supporting information). The two clusters were strongly diverged across the Rio Grande (Fig. 1). The cluster west of the Rio Grande included 18 of 20 sites, and there was a significant pattern of IBD among western sites (Fig. 2; $r = 0.42$, $P < 0.0001$). The STRUCTURE analysis of sites in the western cluster supported subdivision into two additional clusters (Fig. 3, Table S5, Supporting information), which corresponded to the northwestern and southwestern portions of the study area (Fig. 1). Membership coefficients revealed greater admixture when comparing clusters west of the Rio Grande than when comparing clusters separated by the Rio Grande (Figs S1 and S2, Supporting information).

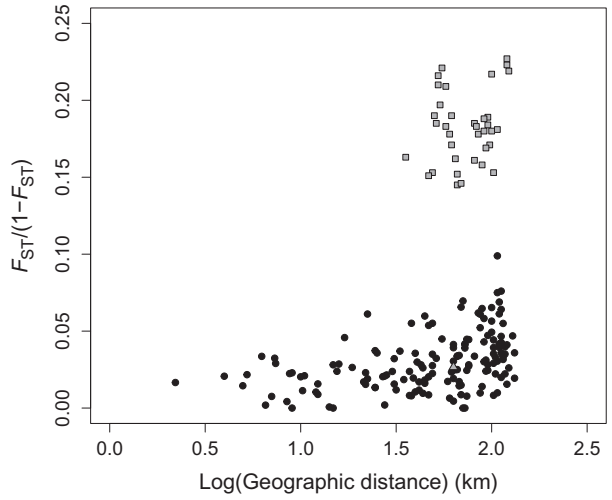

Fig. 2 Relationship of pairwise genetic distances to pairwise geographical distances between 20 populations of *Dipodomys spectabilis* in southern New Mexico, USA. Plot indicates site pairs west of the Rio Grande (black circles), site pairs east of the Rio Grande (grey triangles) and site pairs on opposite sides of the Rio Grande (grey squares).

Genetic response to restoration

At treated sites, treatment age was the most important predictor of allelic richness based on its presence in the top three models with competitive support and an intercept model with ΔAIC > 2 (Table 2). Allelic richness was negatively related to treatment age (Fig. 4; Table 2). Models with connectivity or area were not strongly supported (Table 2). Connectivity was in the most-supported model of expected heterozygosity, but the negative effect of connectivity was marginal based on the competitive support of the intercept-only model (Table 2). Expected heterozygosity was not strongly related to treatment age or area (Table 2). Genetic divergence among recently colonized populations was not significantly different than among established populations (recently colonized F_{ST} = 0.023; established F_{ST} = 0.033; P = 0.73).

Among treated sites west of the Rio Grande, mean allelic richness was 6.26 (SE = 0.08) and mean expected heterozygosity was 0.69 (SE = 0.005). Allelic richness and expected heterozygosity did not differ between treated sites and the remnant site west of the Rio Grande (Site 13; A_R: t = 1.62, d.f. = 16, P = 0.12; H_E: t = 0.52, d.f. = 16, P = 0.61).

Discussion

Despite occurring in a highly fragmented landscape and having a slow demographic response to habitat restoration, our results show no sign of founder effects when *Dipodomys spectabilis* passively colonizes restored grasslands. Allelic richness was greatest early during the restoration trajectory, and heterozygosity did not vary with treatment age. Furthermore, genetic divergence among recently colonized sites was on par with genetic divergence among established sites. Our results do not indicate that founder effects were mitigated by the size or spatial configuration of treated sites. Overall, our results show that genetic restoration can proceed more quickly than demographic restoration. We hypothesize that this outcome is associated with changes in density and dispersal of *D. spectabilis* after shrub removal treatments, but we also discuss alternative explanations that could cause high gene flow early in the restoration trajectory.

Genetic response to restoration

Few studies on animals have demonstrated that landscape-scale habitat restoration can have positive

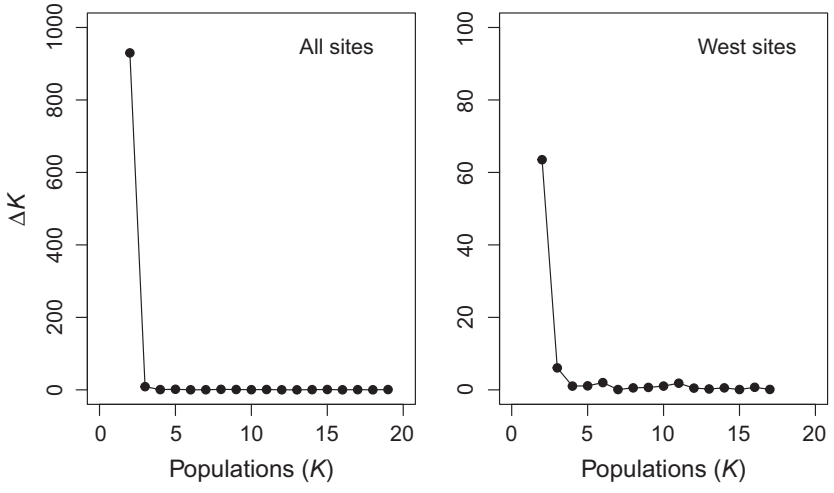

Fig. 3 Second order rate of change for mean log probability between sequential values of the number of populations (Δ*K*) based on the STRUCTURE analysis for all sites and sites west of the Rio Grande. Results based on 15 simulations at each value of *K*.

Table 2 Model selection statistics and beta coefficients (b) for allelic richness (A_R) and expected heterozygosity (H_E) for *Dipodomys spectabilis* at sites treated with herbicide in southern New Mexico, USA. Only treated sites west of the Rio Grande were included (n = 17). Statistics include the difference between AIC of each model and the most-supported model (ΔAIC), Akaike model weight (ω), log-likelihood (LL), and number of parameters (K). Main effects included age of treated site (years since shrub removal), connectivity to source populations and area of treated site.

Response	Model	ΔAIC	ω	LL	K	b_{Age} (SE)	$b_{Connect}$ (SE)	b_{Area} (SE)
A_R	Age	0.00	0.224	−180.43	4	−0.19 (0.09)	–	–
	Age + Connect	0.43	0.180	−179.65	5	−0.16 (0.09)	−0.12 (0.09)	–
	Age + Area	1.01	0.135	−179.94	5	−0.15 (0.10)	–	−0.10 (0.10)
	Connect	1.29	0.118	−181.08	4	–	−0.16 (0.09)	–
	Area	1.55	0.103	−181.21	4	–	–	−0.15 (0.09)
	Connect + Area	1.85	0.089	−180.36	5	–	−0.12 (0.09)	−0.11 (0.09)
	Age + Connect + Area	1.92	0.085	−179.40	6	−0.13 (0.10)	−0.10 (0.09)	−0.07 (0.10)
	Intercept	2.43	0.066	−182.65	3	–	–	–
H_E	Connect	0.00	0.250	118.89	4	–	−0.012 (0.007)	–
	Intercept	0.62	0.184	117.58	3	–	–	–
	Area	1.09	0.145	118.34	4	–	–	−0.009 (0.007)
	Connect + Area	1.42	0.123	119.17	5	–	−0.010 (0.007)	−0.006 (0.007)
	Age + Connect	1.85	0.099	118.96	5	−0.003 (0.007)	−0.011 (0.007)	–
	Age	1.94	0.095	117.92	4	−0.006 (0.007)	–	–
	Age + Area	2.94	0.058	118.42	5	−0.003 (0.008)	–	−0.008 (0.008)
	Age + Connect + Area	3.40	0.046	119.19	6	−0.001 (0.008)	−0.009 (0.008)	−0.005 (0.008)

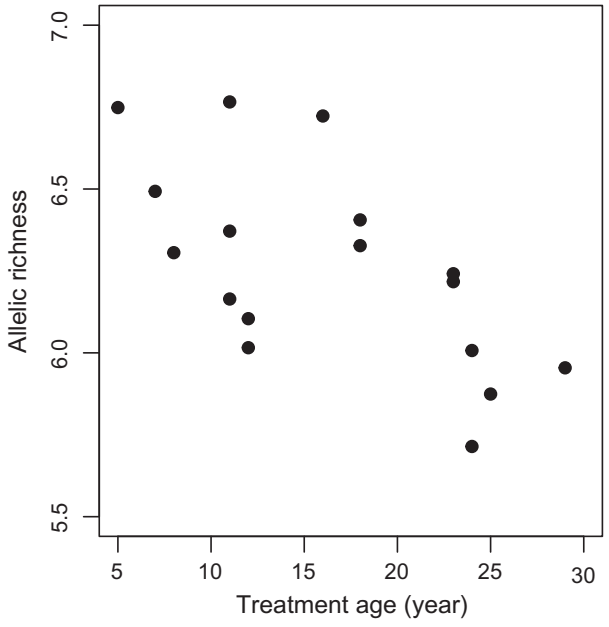

Fig. 4 Relationship of allelic richness for *Dipodomys spectabilis* to age of treated sites (years since shrub removal). Allelic richness is shown as the average across loci for presentation purposes. For analysis, locus was treated as a random effect in a linear mixed model. The generation time for *D. spectabilis* is 1.7 years.

outcomes for demographic and genetic components of biodiversity. Neuwald & Templeton (2013) showed prescribed burning stabilizes metapopulation dynamics and restores genetic diversity in eastern collared lizards (*Crotaphytus collaris collaris*) by increasing gene flow. In our study system, application of herbicide at landscape scales reduces shrub cover and increases perennial grass cover, creating novel savannah ecosystems that are distinct from remnant grasslands (Coffman *et al.* 2014). Despite the difference in habitat structure between treated and remnant sites, restoration efforts increase density of *D. spectabilis* (Cosentino *et al.* 2014) and support levels of genetic diversity comparable to remnant sites (this study). Although our comparison of genetic diversity was limited by the number of remnant sites, levels of genetic diversity at our treated sites were also in line with two remnant populations in southeastern Arizona ('Rucker' and 'Portal'; Busch *et al.* 2007). Busch *et al.* (2007) found allelic richness levels of 6.50–7.37 and observed heterozygosity levels of 0.63–0.73 at Rucker and Portal across multiple years of study.

Allelic richness was similar between treated and remnant sites despite a negative association with treatment age. The disparity in allelic richness between our youngest and oldest restoration sites was not particularly large (~1 allele; Fig. 4). However, even a weak negative association between allelic richness and treatment age indicates the absence of strong founder effects during colonization of treated sites. This conclusion was corroborated by consistent levels of genetic divergence among recently colonized and established populations. Our results suggest that colonization by *D. spectabilis* may approach a migrant pool model in which colonists

are drawn from multiple source populations (Slatkin 1977). Alternatively, founder effects can be avoided if the number of colonists is greater than the average number of migrants among extant populations (Whitlock & McCauley 1990). Regardless of the mechanism, our findings suggest that gene flow overwhelms drift during the initial phase of population growth at treated sites.

High gene flow early in the restoration trajectory is surprising in light of studies suggesting *D. spectabilis* has limited dispersal ability (Jones *et al.* 1988; Waser & Elliott 1991; Skvarla *et al.* 2004; Waser *et al.* 2006). One possible explanation for high gene flow early in the restoration trajectory is negative density-dependent dispersal. Busch *et al.* (2007) proposed the same mechanism to explain the absence of genetic signatures of known demographic bottlenecks, noting that dispersers are seven times more likely to disperse >100 m in low-density years than in medium-density years (Waser *et al.* 2006) and that recolonization commonly occurs after local extinction at one of their sites ('Rucker'; Swanson 2001). Vacant mounds can persist on the landscape ≥50 years (Parmenter & Van Devender 1995). At shrub-encroached sites where shrubs are removed, recruitment of immigrants may be greatest early in the restoration trajectory because density is low and vacant mounds are readily available to be renovated (B. J. Cosentino, personal observation). As density increases, immigration should decline. Thus, genetic drift and gene flow should both weaken as time since treatment increases. The weak negative association between allelic richness and treatment age suggests that gene flow weakens at a slightly greater rate than genetic drift. Although average density increases over time at restoration sites, *D. spectabilis* populations are known to fluctuate stochastically over time in response to rainfall and primary production (Munger *et al.* 1983; Brown & Zeng 1989; Busch *et al.* 2007). Fluctuating population size may cause strong drift to persist even at old restoration sites because only a single generation at small population size is needed to reduce genetic variation (Allendorf *et al.* 2013). However, genetic drift associated with population fluctuations may be partially balanced by occasional gene flow (during density declines) given the weak decline in allelic richness over time and the equivalent levels of genetic divergence for recently colonized and old populations.

Experimental data on temporal changes in density and gene flow are needed to confirm whether negative density-dependent dispersal explains the lack of founder effects at restoration sites. We propose three alternative explanations that are also consistent with the observed patterns. First, long-distance dispersal for *D. spectabilis* could be underestimated. The pattern of IBD we identified suggests *D. spectabilis* gene flow decreases as geographical distance increases, but the magnitude of F_{ST} for pairs of populations on the same side of the Rio Grande indicates only weak divergence (Fig. 2). Parentage analysis and assignment tests indicate previous estimates of dispersal distances were too low for *D. spectabilis* because some offspring disperse before their first capture in mark–recapture studies (Waser *et al.* 2006; Waser & Hadfield 2011). Moreover, long-distance dispersal is underestimated by traditional mark–recapture methods because of finite study areas (Koenig *et al.* 1996). Using reverse time capture–recapture modelling, Sanderlin *et al.* (2012) explicitly examined how dispersal from outside their study area in Arizona affected population growth of *D. spectabilis*. Estimates of immigration from outside the study area were 'unexpectedly large' due to either unaccounted long-distance dispersal or inability to trap late-born young during the summer sampling period (Sanderlin *et al.* 2012).

Second, colonists may originate from areas that have not been treated with herbicide. We assumed that source populations occur primarily in treated areas because high-density populations do not occur in shrubland (Cosentino *et al.* 2014). However, a small number of isolated, occupied mounds can occur in adjacent shrublands (Cosentino *et al.* 2014), and individuals from there may not have to move long distances to colonize treated areas. Some source populations also may occur in small remnant grasslands of which we are unaware, but the extent of grassland loss in the region (Gibbens *et al.* 2005; Yanoff *et al.* 2008) suggests this scenario is unlikely.

Third, we cannot rule out the possibility that populations were depressed but not extirpated before sites were treated for restoration, although our experience indicates this is uncommon. In a study of 18 sites treated with herbicide in 2009–2010, only 1 site had occupied mounds at the time of treatment, whereas all sites had sign of old mounds indicating the presence of a historical population (R. L. Schooley, unpublished data). Moreover, bottlenecks and extirpation followed by recolonization are both processes that should increase the strength of genetic drift. High gene flow would still be needed to explain the high levels of genetic diversity observed at young restoration sites.

In addition to examining changes in genetic structure over time, we assessed whether the size and spatial configuration of treated sites affects the genetic response to restoration. Treatment area was not an important predictor of allelic richness or heterozygosity, but heterozygosity had a marginal negative association with connectivity to source populations. Connectivity also had a negative coefficient in a competitive model

of allelic richness (Table 2). Relationships between genetic diversity and connectivity are often positive (e.g. Keyghobadi *et al.* 2005; Helm *et al.* 2009; Cosentino *et al.* 2012), likely because gene flow is enhanced by connectivity. In our system, gene flow may have been negatively related to connectivity due to the positive association between connectivity and density (Cosentino *et al.* 2014) and the known pattern of negative density-dependent dispersal for *D. spectabilis*. Extensive sampling of treated areas and potential source populations at a fine spatial scale and use of assignment tests to identify immigrants and their source populations would provide greater insight into mechanisms of colonization and the role of spatial connectivity (e.g. Helsen *et al.* 2013, 2015). A landscape genetics approach may also provide insight into the effects of the landscape matrix on gene flow (e.g. McRae *et al.* 2008).

One limitation to our analysis of temporal changes in genetic structure at treated sites was the use of a space-for-time substitution. The immediate genetic response of populations to treatments is unknown because the youngest site in our sample was five years post-treatment (≤3 generations). Furthermore, the youngest sites might not have been representative due to the need to obtain a reasonably large sample of adults for population genetic analyses. Density of *D. spectabilis* at some young sites was low enough to prohibit inclusion in our study. If density at young sites in our sample was greater than average, allelic richness at young sites may have been inflated due to weaker-than-average genetic drift. Longitudinal data from individual sites are needed to clarify temporal changes in genetic structure and provide insight into the roles of density and dispersal in shaping those changes (e.g. Berthier *et al.* 2006; Pilot *et al.* 2010; Gauffre *et al.* 2014).

Population structure

Dipodomys spectabilis was strongly divided into eastern and western genetic clusters (Fig. 1). These clusters suggest the Rio Grande acts as a major barrier to gene flow. This pattern was corroborated by F_{ST} values, which were greatest between populations separated by the Rio Grande (Fig. 2). Among rodents, Sullivan (1994) found the Rio Grande was a phylogeographical barrier for Mexican woodrats (*Neotoma mexicana*). Studies of cactus beetles (*Moneilema* spp.) have also revealed strong genetic divergence across the Rio Grande (Smith & Farrell 2005a,b). Further studies are needed to clarify whether gene flow in *D. spectabilis* was historically restricted by the river, or whether divergence was caused more recently by agricultural land use and roads. Land adjacent to the Rio Grande is heavily used to grow pecans, chili peppers and cotton, and Interstate 25 is parallel to the Rio Grande for the entire north–south length of our study area.

The STRUCTURE analysis revealed additional subdivision into two clusters west of the Rio Grande. Divergence between clusters west of the Rio Grande was much weaker than divergence across the river. There are no obvious geographical features that would limit gene flow between the two clusters. Given that the two sites near the boundaries of their respective clusters were strongly admixed (sites 3 and 8; Fig. S2, Supporting information), the clusters could be due to IBD rather than geographical barriers to gene flow.

Conclusions

The United Nations Conference on Sustainable Development (Rio+20) established a goal of restoring 150 million ha of land by 2020 (Menz *et al.* 2013). Based on our work on *D. spectabilis* in the Chihuahuan Desert, it is exciting to know that landscape-scale restoration efforts can have positive outcomes for demographic and genetic components of biodiversity, even when practitioners rely on passive responses of species to restoration. In particular, our results demonstrate that genetic variation can be quickly restored even when there is a time-lagged demographic response, likely due to gene flow compensating for genetic drift. This finding shows that monitoring programmes may reveal demographic and genetic trajectories that are inconsistent after restoration treatments. One unanswered question is whether landscape restoration efforts maintain adaptive potential in animal populations, particularly as species like *D. spectabilis* face selection pressures imposed by a changing landscape and climate (Moses *et al.* 2012). Microevolutionary responses may be particularly important for mitigating the effects of environmental change for species like *D. spectabilis* that are presumed to have limited ability to disperse long distances.

Importantly, our results demonstrate that genetic founder effects are not inevitable for animals during passive colonization of restoration sites. Lack of strong founder effects during natural colonization has been demonstrated in multiple cases for plants (e.g. Raffl *et al.* 2006; Helsen *et al.* 2013, 2015), but there are fewer examples for animals (but see Dybdahl 1994; Forbes & Boyd 1996; Clegg *et al.* 2002). We know even less about how variation in dispersal characteristics (e.g. density dependence, dispersal distances) among animal species affects the strength of founder effects during colonization. Further study is needed to determine whether dispersal and other life-history traits can be used to predict the likelihood of founder effects to help managers assess risk and the need for mitigation in the context of passive restoration.

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B.J.C., R.L.S. and B.T.B. designed the study. B.J.C., A.J.M. and K.S. collected the data. B.J.C. analysed the data and wrote the manuscript. All authors revised and approved the final manuscript.

Data accessibility

The following data are available on Dryad (doi:10.5061/dryad.gq246):

- Year of sampling, geographical coordinates and treatment status of sites
- Microsatellite genotypes
- Pairwise geographical and genetic distances
- STRUCTURE input files

Supporting information

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

Tables S1–S3 Locus-specific estimates and bias-corrected 95% confidence intervals of observed heterozygosity (H_O), expected heterozygosity (H_E) and allelic richness (A_R) for *Dipodomys spectabilis* at sites in the Chihuahuan Desert of southern New Mexico, USA.

Table S4 Pairwise F_{ST} estimates for *Dipodomys spectabilis* between sites in the Chihuahuan Desert of southern New Mexico, USA.

Table S5 Mean log probability of the data ($L(K)$) for a given number of populations (K) and second order rate of change of

the mean log probability between sequential values of K (ΔK) for all sites and sites west of the Rio Grande.

Fig. S1 Membership of *Dipodomys spectabilis* individuals in clusters inferred from the STRUCTURE analysis.

Fig. S2 Membership of *Dipodomys spectabilis* individuals from sites west of the Rio Grande in clusters inferred from the STRUCTURE analysis.