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Population declines lead to replicate patterns of internal range structure at the tips of the distribution of the California red-legged frog (*Rana draytonii*)

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ABSTRACT

Demographic declines and increased isolation of peripheral populations of the threatened California red-legged frog (*Rana draytonii*) have led to the formation of internal range boundaries at opposite ends of the species' distribution. While the population genetics of the southern internal boundary has been studied in some detail, similar information is lacking for the northern part of the range. In this study, we used microsatellite and mtDNA data to examine the genetic structuring and diversity of some of the last remaining *R. draytonii* populations in the northern Sierra Nevada, which collectively form the northern external range boundary. We compared these data to coastal populations in the San Francisco Bay Area, where the species is notably more abundant and still exists throughout much of its historic range. We show that 'external' Sierra Nevada populations have lower genetic diversity and are more differentiated from one another than their 'internal' Bay Area counterparts. This same pattern was mirrored across the distribution in California, where Sierra Nevada and Bay Area populations had lower allelic variability compared to those previously studied in coastal southern California. This genetic signature of northward range expansion was mirrored in the phylogeography of mtDNA haplotypes; northern Sierra Nevada haplotypes showed greater similarity to haplotypes from the south Coast Ranges than to the more geographically proximate populations in the Bay Area. These data cast new light on the geographic origins of Sierra Nevada *R. draytonii* populations and highlight the importance of distinguishing the genetic effects of contemporary demographic declines from underlying signatures of historic range expansion when addressing the most immediate threats to population persistence. Because there is no evidence of contemporary gene flow between any of the Sierra Nevada *R. draytonii* populations, we suggest that management activities should focus on maintaining and creating additional ponds to support breeding within typical dispersal distances of occupied habitat.

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1. Introduction

Spatial variation in abundance within a species range can lead to internal range structure, with the number, size and location of gaps and fragments tending to increase towards the range edge. This 'globular' nature of a species' internal range can be the result of several processes, including long-range dispersal of a few individuals across one or more biogeographic barriers leading to new isolated populations, the formation of barriers within a continuous distribution to create satellite populations, spatiotemporal variation in the environment, or rapid contraction of a formerly widespread species leaving small isolates in the wake of the contraction (Brown et al., 1996). Internal disjunctions that develop as a result of the latter processes can be a cause for conservation concern, particularly for threatened and endangered taxa, because they have the potential to eliminate important dispersal and genetic resupply routes. The problem may be especially acute if internal structure forms towards the tips of the species range, where edge populations are often already challenged by greater isolation and lower abundance (Sexton et al., 2009).

The threatened California red-legged frog *Rana draytonii* is one such species for which the internal range structure has changed considerably in recent times, and it is widely accepted among herpetologists that the species is in decline (Davidson et al., 2001; Fellers, 2005; Fisher and Shaffer, 1996; Jennings and Hayes, 1985,

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1994; Moyle, 1973; Richmond et al., 2013). Some of the most extreme examples of local extirpation causing shifts in range structure have occurred in southern California – since the 1960s, the species has disappeared from virtually all historical localities spanning from the US–Mexico border northward through the Transverse Ranges, with the exception of a single coastal population that persists in the Santa Monica Mountains to the west/northwest of the Los Angeles Basin. This rapid extirpation has led to the formation of an internal range boundary and a ~500 km distributional gap between the southern-most *R. draytonii* populations in California and six remnant populations in the Sierra San Pedro Martir of Baja California (Fig. 1), where the species also appears to be extirpated from much of its historical range (A. Peralta Garcia, unpub. data).

Extensive local extirpation has also occurred at the southern end of California's Great Central Valley (i.e. Tulare Basin), where *R. draytonii* was commercially harvested as a food resource during the Gold Rush era of the late 1800s and early 1900s (Jennings and Hayes, 1985). Subsequent loss of suitable habitat due to water diversion, agriculture, and extensive urbanization throughout the 1900s has led to the complete disappearance of *R. draytonii* from the southern Valley floor and possibly the southern Sierra Nevada. At the northern end of the Great Central Valley, the species' range has deteriorated in a manner that resembles the patterns in southern California and northern Baja California, but the timing of population declines in this part of the range and the area encompassed by the historical distribution are less clear. Currently, Sierra Nevada populations are no longer connected to coastal populations around the north end or across the Central Valley floor, leaving a detached cluster of small, isolated populations in the Sierra Nevada foothills that geographically resemble the remnant group in the Sierra San Pedro Martir (Fig. 1).

The dissociation of peripherally isolated groups of populations at the northern and southern tips of the *R. draytonii* distribution have eliminated opportunities for dispersal and gene exchange with a larger and more robust 'core' formed by interior populations in the south Coast Ranges of California (U.S. Fish and Wildlife Service, 1996; Fellers, 2005; Jennings and Hayes, 1994). This core extends from the Russian River in Sonoma County to the Santa Ynez Mountains in Santa Barbara County. Although habitat loss has reduced the number of populations within the core distribution, many large populations still persist throughout much of this part of the range (Fellers, 2005; M. Westphal pers. comm.). This higher abundance and presumably greater connectivity among central coast populations likely places them at an evolutionary advantage with respect to marginal populations at the tips of the species distribution, where small population sizes and greater isolation among marginal groups may leave them more susceptible to loss of genetic diversity through drift (Eckert et al., 2008; Kirkpatric and Barton, 1997; Sexton et al., 2009). Stochastic environmental and demographic events may also have more pronounced effects on marginal populations because they cannot be recolonized naturally due to their degree of isolation. These factors ultimately translate to a higher risk of extinction.

While the genetic effects of demographic declines have been studied to some extent at the southern internal range boundary in California (Richmond et al., 2013), very little genetic data exists from northern *R. draytonii* populations for comparative analysis (but see Pauly et al., 2008; Shaffer et al., 2004). Current data suggest that the species occurs at only seven of its 26 historically

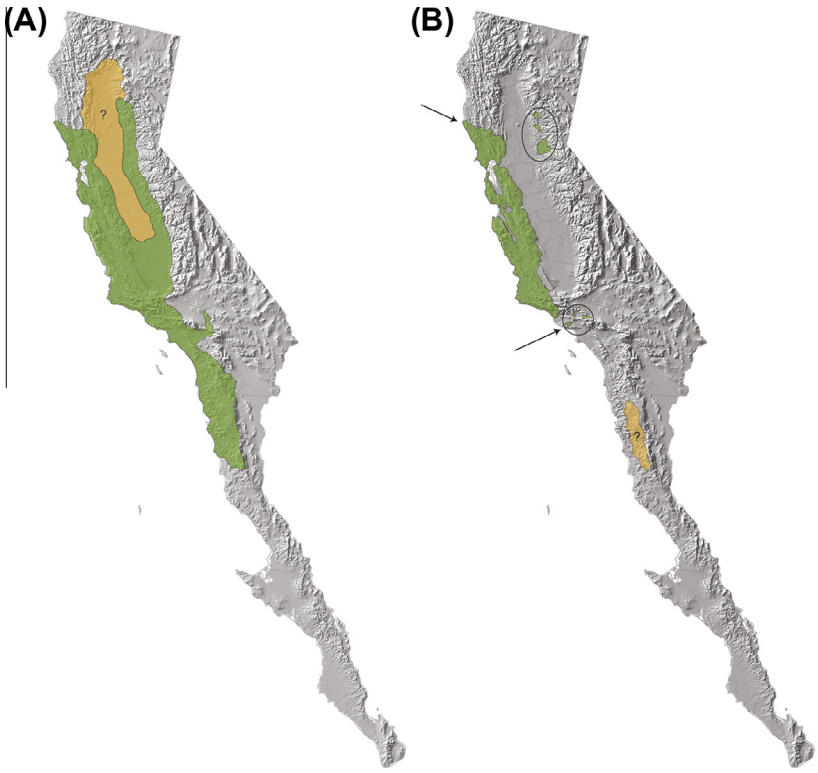

Fig. 1. Approximate distribution of the historical (A) and contemporary range (B) of *Rana draytonii*. Orange polygons indicate uncertainty about the distribution. Dark ovals highlight marginal populations in the northern Sierra Nevada and southern California; arrows denote internal range boundaries. Little is known about the extent of the historical distribution of *R. draytonii* on the Central Valley floor, particularly for the mid to northern end (i.e. delta region, the Sacramento Valley and the San Joaquin Valley); however, Jennings and Hayes (1985) cite historical notes claiming that commercial amounts of *R. draytonii* were harvested from the southern end of the Central Valley (Tulare Basin) prior to bullfrog introductions. This end of the Valley contained the largest freshwater lake west of the Mississippi River and an extensive mosaic of marshes and sloughs as recently as the late 1800s.

known localities in the Sierra Nevada, less than half of which have more than 20 adults (Barry and Fellers, 2013; USFWS, 2008). To assist in developing recovery strategies for these Sierra Nevada populations, we use data from microsatellites and mitochondrial DNA (mtDNA) sequences to begin characterizing their landscape genetic structure and diversity. We compare these data to coastal populations in the San Francisco Bay Area, where *R. draytonii* are considerably more abundant and form the northern internal range boundary of the core distribution. Our main objectives were to compare the degree to which microsatellite alleles and mtDNA haplotypes are shared among individuals across ponds (i.e. genetic admixture), the extent of historical (mtDNA) and contemporary (microsatellites) gene flow, and the genetic diversity among the sampled populations within and between the Sierra Nevada and San Francisco Bay area regional groups. We were particularly interested in testing for central-marginal trends in diversity and differentiation, where Sierra Nevada populations were considered 'external' and San Francisco Bay area populations were considered 'internal'. By internal, we simply mean that Bay Area populations are geographically closer to the core distribution in the south Coast Ranges, and that unlike the Sierra Nevada, the species is relatively common in the Bay Area.

2. Materials and methods

2.1. Field sampling

We obtained property access to sample 75 *R. draytonii* from five locations spanning four counties in the northern Sierra Nevada: Hughes Pond, Sailor Flat, Big Gun Diggings, Bear Creek, and Spivey Pond (Fig. 2). Sampling took place using dip nets and frogs were released on site after tissue sampling. Tissue samples consisted of small toe clips taken from the distal outer phalange on the right forelimb and were immediately placed in 95% ethanol. When insufficient numbers of adults or subadults were present, we clipped the distal tip (<5 mm) of the tail from tadpoles.

We also used tissues ($N = 35$) obtained from field surveys in the San Francisco Bay Area and Point Reyes (Fig. 1). These samples represented populations from five different locations, three on the north side of the San Francisco Bay inlet in Marin County (Rodeo Lake, Point Reyes Peninsula [P-72] and Olema Valley [P-84]) and two on the south side in San Mateo County (Mori Point and Milagra Ridge; Fig. 2). Tissue samples were collected under the appropriate permits and guidelines issued by the U.S. Fish and Wildlife Service and the California Department of Fish and Wildlife – copies are available upon request.

2.2. DNA extraction and lab protocol

We extracted genomic DNA using a Qiagen® DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA) and collected information for two data sets, one consisting of mitochondrial gene sequences and the second consisting of allele frequency data from 15 microsatellite loci. The microsatellites were developed specifically for *R. draytonii* in a previous study using DNA samples from populations in southern California (Richmond et al., 2013) – primer sequences and a multiplex PCR protocol for microsatellite amplification are available in that study. All PCR products were analyzed

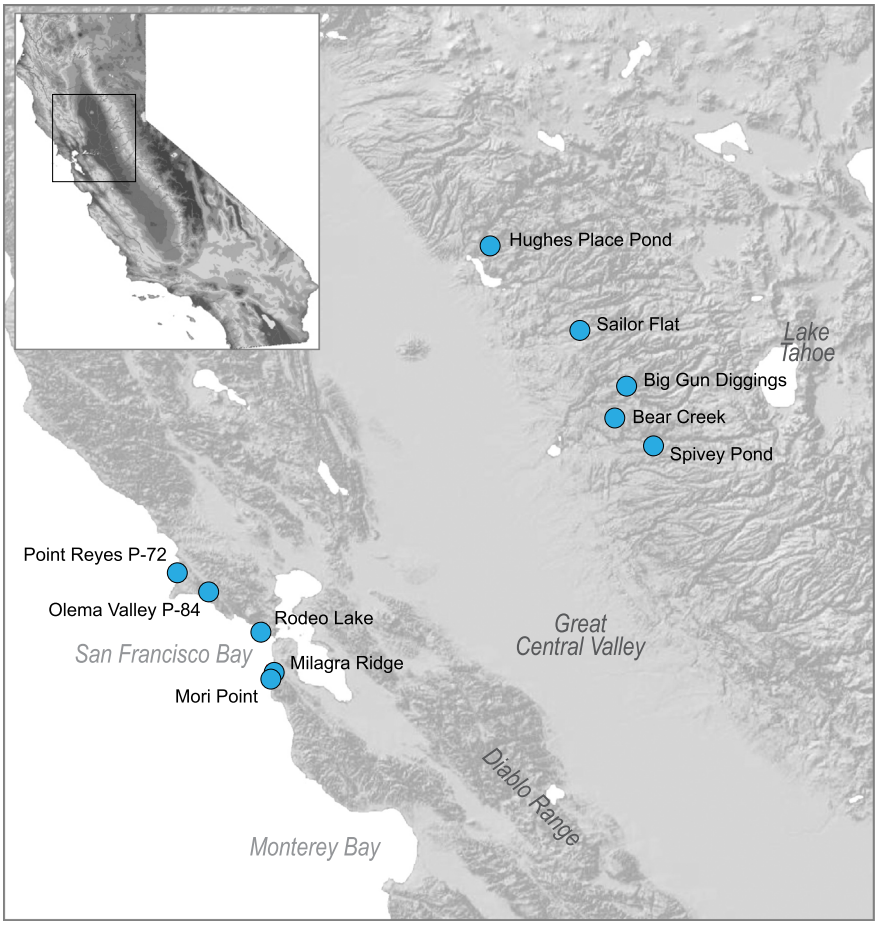

Fig. 2. Sampling locations for this study. Our samples for the Sierra Nevada represent five of the seven known breeding populations of *R. draytonii* for this portion of the range.

on an ABI3730xl DNA analyzer at Bio Applied Technologies Joint, Inc. (San Diego, CA). We edited and scored the raw allelic data in Gene-Marker v1.90 (SoftGenetics).

For mtDNA sequences, we PCR amplified a 1013 base pair fragment of the cytochrome *b* gene using primers MVZ15-L (Moritz et al., 1992) and CytbAR-H (Goebel et al., 1999). We sequenced this region because it overlaps with the same fragment used to good effect in previous phylogeographic studies on *R. draytonii* and *Rana aurora* (Pauly et al., 2008; Richmond et al., 2013; Shaffer et al., 2004) – details on the PCR protocol and primer sequences are outlined in these papers. At least two frogs per sampling location were sequenced, and these data were combined with the dataset for southern California populations (presented in Richmond et al., 2013) for phylogeographic analysis. DNA sequencing was performed using Sanger methods and Big Dye v3.1 chemistry on an ABI3730XL DNA analyzer at Genewiz (La Jolla, CA).

2.3. Data analysis: spatial genetic structuring

We performed cluster assignment tests in Structure v2.3 (Falush et al., 2003; Pritchard et al., 2000) based on the allele frequency data only to generate membership assignment coefficients for all individuals. We then used the cluster assignments to infer spatial patterns in the distribution of genotypes. Membership assignments were evaluated across a range of different numbers of clusters ($K = 2$–10) to which individuals could be assigned. For each K, we performed 10 separate runs using an uncorrelated allele frequency model with admixture (250,000 steps with a burn-in of 100,000 steps). To approximate the number of clusters in the full sample, we used Structure Harvester (Earl and vonHoldt, 2012) to calculate ΔK (Evanno et al., 2005) and plotted the mean $\ln P(D|K)$ score for each of the 10 runs against K, where the number of clusters was based on the asymptote of the $\ln P(D|K)$ curve. We summarized the 10 runs at each K value by generating alignments of the assignment coefficient matrices across runs using the GREEDY algorithm in ClumpP v1.1.2 (Jakobsson and Rosenberg, 2007). We then visualized the alignments as assignment plots using the software Distruct v1.1 (Rosenberg, 2004).

We compared spatial patterns in the microsatellites with cytochrome *b* haplotypes by estimating a phylogenetic tree in BEAST v1.7 (Drummond and Rambaut, 2007). This dataset included several haplotypes from Richmond et al. (2013) that fall within different regional clades in the southern part of the species' range in California. We used Bayesian Information Criterion (BIC) scores generated in jModeltest 2.1.2 (Posada, 2008) to identify a best-fit model of nucleotide evolution and ran the Markov Chain Monte Carlo simulations in BEAST for 2×107 steps, sampling from the posterior every 1000th step. We discarded the first 10% of the samples from the posterior and assessed convergence and effective sample sizes for each model parameter in Tracer v1.5 (Drummond and Rambaut, 2007). BEAST requires the user to select an appropriate demographic tree prior – we explored results using three different priors (Bayesian Skyline, expansion growth, and constant size) to test for possible effects on branch support values and branch length estimates. Consistency in these parameter estimates, regardless of the tree prior, would indicate that the results were not biased by the choice of the prior. We also constructed a haplotype network in TCS v1.21 (Clement et al., 2000) to better visualize the mutational steps separating the different haplotypes.

2.4. Data analysis: genetic diversity and differentiation

We estimated standard genetic diversity estimates for the microsatellites and the mtDNA gene sequences. For microsatellites, we calculated allelic richness (A_R; adjusted for sample size) in HP-Rare 1.0 (Kalinowski, 2005), and observed heterozygosity (H_O), expected heterozygosity (H_E), and Queller and Goodnight's relatedness index (R) in Genalex 6.0 (Peakall and Smouse, 2006). To statistically test for differences in genetic diversity between the Sierra Nevada and San Francisco Bay area populations, we used Fstat (Goudet, 2001) to perform permutation tests using A_R, H_O, H_E, and R (10^3 permutations). Statistical significance ($\alpha = 0.05$) was calculated as the fraction of permuted values that were at least as extreme as the original estimates, which were derived from the original data. We estimated effective population size N_E using an approximate Bayesian method in OneSamp (Tallmon et al., 2008) – for these analyses, we specified a relatively a non-informative prior on N_E (5–500) based on estimates from Richmond et al. (2013) and from our own field observations at the time of sampling.

We calculated pairwise estimates of Wier and Cockerham's F_{ST} in Arlequin 3.5 (Excoffier et al., 2005) to measure population differentiation because it is unbiased with respect to sample size (Wier and Cockerham, 1984). We report significance using two methods that account for multiple tests; the highly conservative Bonferroni test and a 'false discovery rate' approach known as the Benjamini–Yekutieli (B–Y) method (reviewed in Narum, 2006). We used this same F_{ST} measure to perform an analysis of molecular variance using an Infinite Allele Model in GenoDive (Meirmans and Van Tienderen, 2004) – we partitioned the data by regional groups (i.e. Sierra Nevada vs. Bay Area), populations within regional groups, and among individuals within populations.

To test for genetic isolation by distance (IBD), we performed a standard Mantel test (Mantel, 1967) and a 'stratified' Mantel test, the latter of which accounts for hierarchical population structure in the data (Meirmans, 2012). For the stratified Mantel test, we treated the Bay Area and Sierra populations as separate strata because we assumed that the large distances separating these two main groups of samples would artificially inflate the IBD signal, whereas parsing the data in this manner would provide information at a more appropriate geographic scale. We performed 10^3 permutations on a pairwise F_{ST} matrix and the corresponding geographic distances separating collection sites (in meters) to evaluate significance.

To compare mitochondrial haplotype diversity between Sierra Nevada and coastal populations, we used DnaSP v5.10 (Librado and Rozas, 2009) to calculate nucleotide diversity, average number of nucleotide differences, and number of polymorphic sites for each group, where each group consisted of pooled samples from either Bay Area or Sierra Nevada sampling locations. We performed this same set of analyses to compare haplotype diversity between the northern and southern regional groups for the full species range using data from Richmond et al. (2013) for southern California populations.

3. Results

3.1. Spatial genetic structuring: Microsatellites

Cluster assignments showed strong regional specificity, where frogs from each sampling location were incrementally parsed into distinctive clusters as the data were forced to fit successively higher K values (Fig. 3). This 'deconstruction' followed expectations based on geography; the more geographically proximate samples tended to cluster at lower K, but those associations dissipated at higher K. At the lowest K tested ($K = 2$), Sierra and Bay Area frogs assigned to separate clusters with high probabilities. *Ad hoc* estimation of the number of clusters indicated seven among the 11 sampling locations. However, because the number of clusters is not known without error and informative historical patterns emerge from the data at different K values (e.g. founder events),

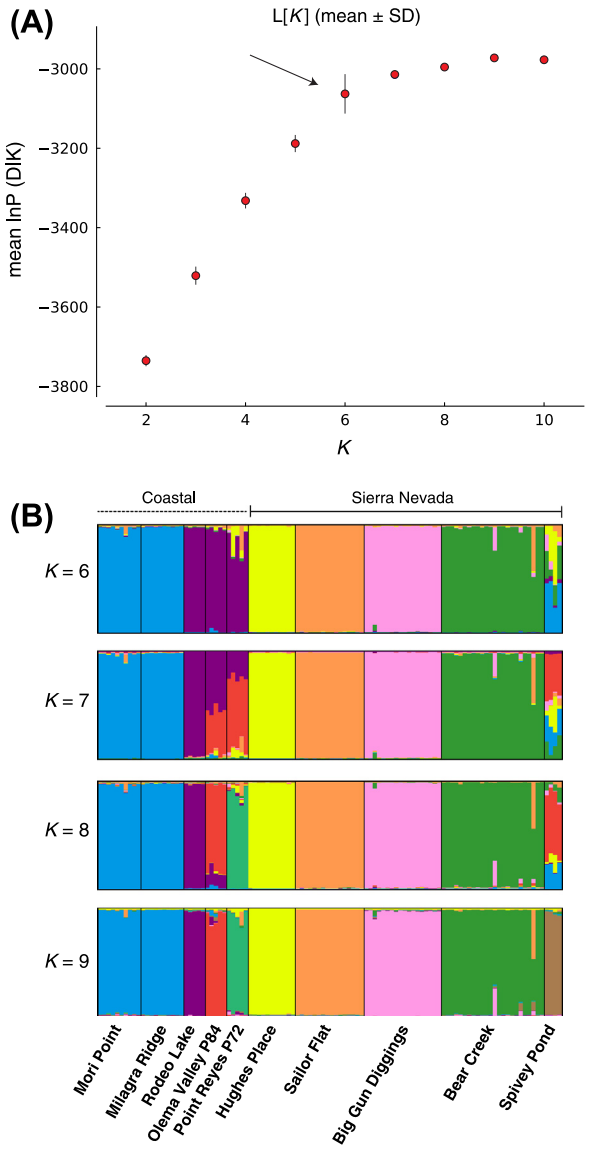

Fig. 3. (A) Plot of the mean ln*P*(D/*K*) scores and standard error bars for each of the 10 Structure runs against different *K* values (arrow approximates the number of clusters). (B) Assignment plots for *K* = 6–10 based on an uncorrelated allele frequency model with admixture.

we present and discuss results around the estimated 'optimal' value (Fig. 3).

Each Sierra population formed a cluster at *K* ranging from five to 10, with the exception of Spivey Pond, which was admixed until *K* = 9. A few frogs from Bear Creek displayed at least some shared assignment with Sailor Flat and Big Gun Diggings, but most individuals were highly distinctive. The shared assignment for these few frogs at Bear Creek and Big Gun Diggings was mirrored in mtDNA haplotypes, where we recovered the same haplotype from both locations and Spivey Pond (see 'Spatial genetic structuring: mtDNA' below). For Bay Area sites, frogs sampled from different parts of Marin County became increasingly more distinctive at higher *K*, with Rodeo Lake showing the greatest exclusivity at lower *K*. In contrast, frogs from Milagra Ridge and Mori Point formed a common cluster across the full range of *K* values tested.

3.2. Spatial genetic structuring: mtDNA

BIC scores based on a maximum likelihood tree topology identified HKY + I as an appropriate substitution model for the cyt*b* sequences. The summary tree from the BEAST analysis showed that Bay Area and Sierra Nevada populations form two well-supported clades (*PP* = 0.99 and 1.00, respectively; Fig. 4a). Surprisingly, Sierra haplotypes were more similar to haplotypes from the south Coast Ranges of northern Santa Barbara County than to the more geographically proximate San Francisco Bay Area populations. This result is supported with high posterior probability (*PP* = 0.97). Bay Area haplotypes also formed a well-supported clade (*PP* = 0.99) that was sister to the 'Sierra Nevada + south Coast Range' clade. These three regional clades together formed a monophyletic group (*PP* = 0.97; Fig. 4a). Use of different tree priors had no discernable effect on tree topology, branch support, or branch length estimates.

The network in Fig. 4b provides a different visualization of the number of mutational steps separating the different haplotypes. A group of haplotypes from the south Coast Ranges (i.e. northern Santa Barbara County) was nested in between the Sierra Nevada and San Francisco Bay area haplotypes, indicating fewer mutational steps separating the Sierra Nevada and south Coast Range haplotypes. The most common Sierra Nevada haplotype was connected to two additional haplotypes (also Sierran), each being only one mutational step away. The Bay Area haplotypes were slightly more differentiated from one another, with two steps at most separating each haplotype. However, so few changes distinguished the different Bay Area haplotypes that any one of several parsimonious reconstructions was possible.

3.3. Genetic diversity and differentiation: Microsatellites

We found no correlation between the number of samples at a given site and the observed number of alleles across loci (Spearman $\rho = -0.15$, d.f. = 9, $P = 0.33$). Despite the larger sample sizes, Sierra Nevada populations tended to have lower numbers of monomorphic loci compared to coastal populations (Table 1), with over one-third of the 15 loci being fixed for a single allele at Hughes Pond and Sailor Flat. When we pooled samples by regional group (Bay Area vs. Sierra Nevada), we found that allelic richness A_R, observed heterozygosity H_O and expected heterozygosity H_E were all significantly higher in the coastal group (Table 2). Queller and Goodnight's index of among-individual relatedness *R* was notably higher in the Sierra Nevada (although not statistically significant based on the permutation test), suggesting that individuals within a pond tend to be more closely related to one another compared to those in coastal ponds. Sierra Nevada populations also showed a higher degree of differentiation among populations versus those on the coast. The AMOVA using an infinite allele model showed that within individual variation accounted for 58% of the total variation in the dataset ($F_{IT} = 0.42$; 95% CI = 0.37–0.48), followed by inter-population variation within the Sierra Nevada and Bay Area groups (33%, $F_{SC} = 0.36$; 95% CI = 0.33–0.39). Only 8% of the total variation was explained by differences among the Sierra and Bay Area groups.

Pairwise F_{ST} values between sampling locations were high for all comparisons, ranging from a low of 0.12 between Mori Point and Milagra Ridge to a high of 0.61 between Sailor Flat and Milagra Ridge (Table 3). All pairwise comparisons showed significant differentiation after correcting for multiple tests using the B–Y method, with fewer significant differences shown for the more conservative Bonferroni test. A standard Mantel test comparing the pairwise F_{ST} and corresponding geographic distance matrices showed a significant signal of IBD (Mantel's $r = 0.32$; $P = 0.002$), as expected for frog populations sampled on either side of the Central Valley; however, when we accounted for population structuring in the data using the stratified Mantel test, the results became non-significant (Mantel's $r = 0.32$; $P = 0.142$).

Estimates of effective size N_e indicate that Sierra populations are quite small, ranging from as few as 8–12 frogs at Hughes Pond

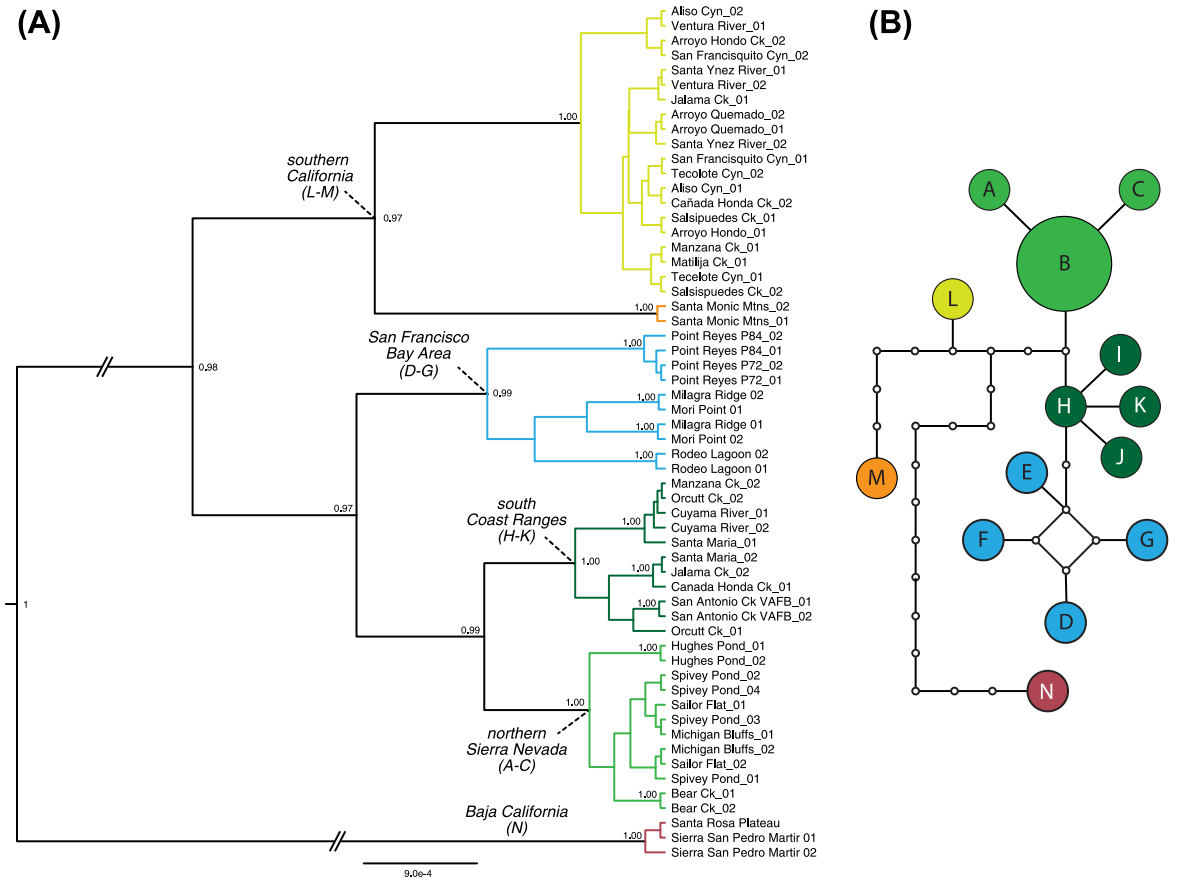

Fig. 4. (A) Maximum clade credibility (MCC) tree from the BEAST analysis. Numbers on the branches indicate posterior probabilities >0.95. Colored branches denote haplotypes corresponding to different regional clades, and letters below the clade names correspond to the haplotype identifiers in the network. Frogs from some sampling locations have haplotypes belonging to both the southern California and south Coast Range clades. (B) Haplotype network with a 90% connection probability limit. Haplotype colors correspond to the MCC tree branches.

Table 1
Diversity indices by sampling location. Notations are as follows: sample size (N), number of monomorphic loci (L_M), mean number of observed alleles across all loci (A), allelic richness corrected for sample size (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), and the 95% credible interval for effective population size (N_E). (Milgra, 9.56–14.30: Mori Point, 9.17–14.21). We did not estimate N_E for sampling locations with fewer than 10 individuals.

Site	N	L_M	A	A_R	H_O	H_E	N_E
Mori Point	10	2	3.29	2.81	0.49	0.51	–*
Milagra Ridge	10	0	2.71	2.19	0.48	0.46	18.00–32.16
Rodeo Lake	5	2	2.43	2.46	0.51	0.48	–
P84	5	2	3.00	2.85	0.45	0.52	–
P72	5	0	3.71	3.46	0.64	0.66	–
Hughes Pond	10	5	2.14	1.91	0.30	0.29	7.48–11.72
Sailor Flat	17	6	1.79	1.47	0.23	0.21	15.83–26.91
Big Gun Diggings	18	3	2.86	2.27	0.42	0.44	23.16–39.61
Bear Creek	24	1	3.00	2.44	0.41	0.46	19.67–41.23
Spivey Pond	4	3	2.29	2.15	0.48	0.46	–

* We combined the data from Mori Point and Milgra Ridge to estimate N_E because the assignment tests in Structure consistently grouped frogs from these sites into a single cluster.

to as many as 41 at Bear Creek. We combined samples from Milagra Ridge and Mori Point to estimate N_e given that the cluster assignments consistently grouped frogs from these two locations across the full range of K tested in the Structure analyses (Table 1). Combining these two samples produced an N_e estimate with credible intervals that overlapped those of the larger Sierra Nevada populations.

3.4. Genetic diversity and differentiation: mtDNA

Of seven unique haplotypes recovered for the Sierra Nevada and Bay Area groups, only 11 of 957 nucleotide sites were variable between the two. Three of the seven haplotypes were exclusive to the Sierra Nevada, one of which was recovered from two-thirds of the frogs from three separate ponds (Hap-B; Supplementary Table S1). Nucleotide diversity, average number of nucleotide differences, and number of polymorphic sites were higher in the Bay Area group, similar to the diversity estimates for microsatellite markers (Table 2). Several sampling locations had haplotypes found exclusively at those locations (Hughes Pond, Bear Creek, and Rodeo Lake), whereas three other haplotypes were shared among frogs in different ponds (although we recognize that more sampling may have revealed additional haplotypes within the monotypic ponds).

When we compared mtDNA haplotype diversity between all northern samples in this study with southern samples from Santa Barbara County south into Baja California, we found that the number of mutations in the southern samples nearly tripled those in the northern samples (27 vs. 11, respectively). Similarly, the average number of nucleotide differences among haplotypes was notably higher in southern individuals ($k = 5.56$ vs. 3.46). However, much of this pattern was driven by the inclusion of samples from a now-extirpated population from the Santa Rosa Plateau (Riverside Co., CA) and from a population in the Sierra San Pedro Martir of northern Baja California. When we excluded these samples from the analysis, the total number of mutations in the

Table 2
Comparison of genetic diversity and differentiation by regional group, arranged geographically from north to south. Data for Santa Barbara/Ventura Co. and 'Southern marginal' populations are from Richmond et al. (2013). 'Southern marginal' refers to an isolated group of populations now forming the southern internal range boundary in California; Santa Barbara/Ventura Co populations form the southern portion of the core species distribution in the south Coast Ranges. Non-shaded columns refer to microsatellite data; shaded columns refer mtDNA sequences. Notations are as follows: allelic richness (A_R), observed heterozygosity (H_O), gene diversity (H_S), Queller and Goodnight's among-individual relatedness (R), pairwise population differentiation within groups (F_{ST}), number of polymorphic nucleotide sites p, average number of nucleotide differences k, and nucleotide diversity π. The asterisk indicates significant differences based on a permutation test in FSTAT.

Group	A_R*	H_o*	H_S*	R	F_{ST}	p	k	π
Sierra Nevada	4.80	0.36	0.38	0.57	0.40	2	0.61	0.001
San Francisco Bay Area	7.02	0.51	0.51	0.42	0.27	6	2.67	0.003
Santa Barbara/Ventura Co.	6.64	0.56	0.56	0.33	0.20	8	3.17	0.003
Southern marginal	4.46	0.44	0.43	0.56	0.38	6	3.20	0.003

Table 3
Pairwise F_{ST} estimates by population. Values in bold were significantly differentiated after B–Y and Bonferroni correction for multiple tests; non-bold values were above the critical value after Bonferroni correction ($\alpha = 0.001$), but below the critical for the B–Y method.

	Mori Pt.	Milagra Ridge	Rodeo Lake	P84	P072	Hughes Pond	Sailor Flat	Michigan Bluff	Bear Creek	Spivey
Mori Pt.	–	**0.12**	**0.32**	**0.27**	**0.27**	**0.38**	**0.53**	**0.37**	**0.30**	**0.22**
Milagra Ridge		–	**0.36**	**0.31**	**0.32**	**0.44**	**0.61**	**0.42**	**0.38**	0.30
Rodeo Lake			–	0.30	0.26	**0.54**	**0.59**	**0.40**	**0.35**	0.36
P84				–	0.22	**0.44**	**0.56**	**0.35**	**0.32**	0.27
P072					–	**0.36**	**0.49**	**0.31**	**0.30**	0.21
Hughes Pond						–	**0.63**	**0.44**	**0.43**	0.41
Sailor Flat							–	**0.47**	**0.36**	**0.57**
Michigan Bluff								–	**0.26**	**0.32**
Bear Creek									–	**0.25**
Spivey Pond										–

southern samples dropped to 13, and the average number of nucleotide differences among haplotypes fell to 3.47.

4. Discussion

Our findings suggest that on a local geographic scale, *R. draytonii* populations in the Sierra Nevada are persisting at low levels of genetic diversity and have no contemporary gene flow across ponds. At the same time, recent range contraction on a larger geographic scale has left a substantial gap between Sierra Nevada and Coast Range populations, similar to the gap separating populations in southern California from the Sierra San Pedro Martir of Baja California. Yet despite these signals of decline, underlying genetic signatures of these same populations still retain evidence of a species that has undergone historical range expansion. These data draw attention to the interplay of contemporary and historical process in determining population structuring, and will ultimately influence how the species is managed in different parts of the range. They also cast new light on the historical biogeography of *R. draytonii* in California and highlight the severity of recent changes in range shape, size, and internal configuration.

4.1. Population structuring and historical phylogeography

Not surprisingly, we found that San Francisco Bay Area and Sierra Nevada *R. draytonii* populations form distinctive groups, and in nearly all cases, frogs clustered uniquely by sampling location. Numerous amphibian studies have shown that population structuring and differentiation is commonly detected at fine geographic scales, sometimes on the order just a few kilometers (Funk et al., 2005; Jehle et al., 2005; Monsen and Blouin, 2003; Shaffer et al., 2000). We observed essentially no admixture across ponds, with the exception of Milagra Ridge and Mori Point, two individuals at Bear Creek, and Spivey Pond. The common clustering of Mori Point and Milagra Ridge individuals across all K values suggests that gene exchange is ongoing between these sites, or that it has only been recently disrupted. Frogs at both of these sites also shared two different mtDNA haplotypes. In fact, Mori Point and Milagra Ridge were the only two sampling locations where different haplotypes were found in frogs at the same location, although greater within-pond sampling may have revealed more heterogeneity in haplotypes.

The two frogs at Bear Creek with slightly admixed genotypes showed similarity to frogs from Sailor Flat and Big Gun Diggings (Fig. 2.), with the latter two sites having a common mtDNA haplotype also recovered at Spivey Pond. The presence of this shared haplotype indicates former connectivity among all of these ponds, although we are limited in how we can interpret the data from Spivey based on only four samples. Nonetheless, both the mtDNA and microsatellite data suggest that the Spivey Pond population is of mixed origin. Increased sampling at this site, combined with a population size assessment and more data from an additional set of genetic markers, would help shed more light on this finding. Given the rarity of *R. draytonii* in the Sierra Nevada and the possibility that these frogs have a composite genetic background, further studies (both demographic and genetic) on the Spivey Pond population as a potential source for translocations are warranted.

Perhaps our most surprising result was that northern Sierra Nevada mtDNA haplotypes were more similar to haplotypes from the southern Coast Ranges than to haplotypes from the more geographically proximate populations in the Bay Area. Based on field notes ($N < 5$) and a small number of museum records ($N < 7$) from the foothills of Butte, Tehama, Shasta, and Glenn Counties, the historical distribution of *R. draytonii* is presumed to have extended around the northern end of the Great Central Valley, linking populations in the northern Sierra Nevada to the San Francisco Bay Area (see range maps in Altig and Dumas, 1972; Jennings and Hayes, 1994; Pauly et al., 2008; Shaffer et al., 2004). Additionally, a few historical records ($N < 7$) exist from the Central Valley in Butte, San Joaquin, Merced, and Fresno Counties, more than 10 miles from the foothills of the Sierra Nevada or the Coast Ranges. This suggests that more direct connections may have also been available across the Valley floor. Our results provide evidence that the expansion into the Sierra Nevada originated from the south Coast Ranges and occurred across the southern portion of the Valley, rather than around or across the northern end.

Trans-Valley patterns have been detected in a variety of species, including other amphibians. In salamanders, *Batrachoseps*

attenuatus (Martinez-Solano et al., 2007) and *Ensatina eschscholtzii xanthoptica* (Wake, 1997) both show historical trans-valley connections at latitudes roughly corresponding to the Bay Area, and that dispersal likely proceeded from west (Coast Ranges) to east (Sierra Nevada). In the foothill yellow-legged frog *Rana boylii*, a central coast mitochondrial clade also includes haplotypes from the northern Sierra Nevada foothills in Yuba County (Lind et al., 2011), a pattern strongly resembling our results here for *R. draytonii*. These and other examples of trans-Valley historical connections (e.g. *Antrodiaetus* mygalomorph spiders (Hedin et al., 2013); *Neotoma* woodrats (Matocq, 2002); *Emys* turtles (Spinks et al., 2010); *Aliatypus* trapdoor spiders (Satler et al., 2011) suggest that the pattern detected here for *R. draytonii* is not that unusual. In direct support of the trans-Valley hypothesis, Jennings and Hayes (1985) concluded that *R. draytonii* were commercially harvested in Kern and Tulare counties in the southern Central Valley prior to 1890, suggesting that populations were once stable enough on the Valley floor to permit interchange between the Coast Ranges and the Sierra Nevada.

We note two caveats regarding the trans-valley hypothesis for *R. draytonii* given the current data. First, we lack samples from a substantial portion of the south Coast Range in California, particularly the Diablo Range, where stronger signatures of cross-valley gene exchange may be present. Second, additional genetic markers are needed to provide more convincing evidence of this interchange, as the cyt*b* haplotypes represent a single, maternally inherited locus that is separate from the nuclear genome. We are currently pursuing a double digest RADseq approach (Peterson et al., 2012) to isolate short sequence fragments with single nucleotide polymorphisms (SNPs) for population representatives throughout the species range. This newly developed next-generation sequencing approach will allow us to generate tens of thousands of SNP markers scattered throughout the genome, and with the inclusion of samples from the south Coast Ranges and Baja California we will be able substantially increase the resolution of the data by sequencing only a few individuals per location.

4.2. Center–margin trends in diversity and differentiation

Sierra Nevada populations had lower allelic diversity, greater inter-individual relatedness within ponds, and greater differentiation among ponds than their Bay Area counterparts. We recovered a similar pattern in the mtDNA, where all diversity indices were higher for the Bay Area group. These findings suggest an increased tendency for breeding among closely related individuals within Sierra Nevada ponds and a potentially accelerated loss of genetic diversity through drift. At the same time their isolation restricts opportunities for migration and gene exchange, as shown by the high F_{ST} values. Similar center-margin trends were detected for *R. draytonii* in southern California, where marginal populations in Los Angeles and Ventura Counties were found to be genetically depauperate and more differentiated compared to coastal populations further north in Santa Barbara County (Richmond et al., 2013). Thus, data from both ends of the species distribution in California suggests that reduced intra-population diversity and greater inter-population differentiation is associated with proximity to the range edge.

Our ability to infer a center-margin trend in effective population size N_E was hampered by small sample sizes for the Bay Area populations and reduced numbers of polymorphic loci for others. Tallmon et al. (2008) suggested that samples of 20 or so individuals and 10 or more polymorphic loci should generate reliable N_E estimates using the OneSamp approximate Bayesian approach. Thus we emphasize caution for estimates based on sample sizes below this threshold, except in cases where we are confident that the actual population sizes are likely fewer than 20 individuals (e.g. some Sierra Nevada populations: Barry and Fellers, 2013; this study). At Hughes Pond for example, we were confident that we captured nearly all of the frogs in the pond during our sampling effort ($n = 10$). Our N_E estimates for a handful of samples were consistent with *R. draytonii* populations in southern California and other *Rana* species in the Pacific Northwest and elsewhere, where effective population sizes of a few tens of individuals tends to be the norm (Ficetola et al., 2010; Phillipsen et al., 2011; Richmond et al., 2013; Schmeller and Merilä, 2007; Schoville et al., 2011).

Aside from effective population size, diversity and differentiation estimates between Bay Area and Sierra Nevada populations are consistent with the genetic predictions of the so-called 'abundant center model', which postulates that individual fitness is highest under certain conditions where the species is most abundant and lower in areas that deviate from that optimum (Brown, 1984; Hengeveld and Haeck, 1982). This should translate to larger and more highly connected populations toward the interior of the species range, and smaller and more isolated populations towards the range edge (Vucetich and Waite, 2003). A simple extension of this ecological model is that genetic diversity and gene flow decline with proximity to the range edge (Eckert et al., 2008; Howes and Lougheed, 2008). Population studies conducted at both ends of the *R. draytonii* distribution in California now show combined support for this model, and suggest that declines in genetic diversity and increased isolation of marginal populations may be contributing to the dissipation of both internal (i.e. southern California) and external range edges (i.e. Sierra Nevada).

4.3. General range-wide diversity trends

Several patterns in the microsatellite and mtDNA data became apparent when we compared the northern samples from this study with those from southern California. Most noticeable was the lower allelic variability in the northern samples, with several microsatellites showing essentially no polymorphism across sampling locations. This lack of polymorphism was not randomly distributed across loci; for example, RADR3-01 was monomorphic in all but two populations and RADR3-02 was monomorphic in all but one. These same markers were among the least variable in Richmond et al. (2013), yet they still contained enough information to be spatially informative for southern California populations. This north–south discrepancy in genetic variability is commonly observed in species that have undergone northward range expansion following post-Pleistocene glacial retreat (Green et al., 1996; Hewitt, 1996, 2004; Howes et al., 2006). This is because populations at the expanding range front are expected to carry only a limited subset of the variation present in the 'trailing' populations from which they were expanding. If low polymorphism in RADR3-01 and RADR3-02 were reduced even further during the course of range expansion, it is entirely plausible that northern populations diverged without having any allelic variability at these loci to begin with.

Excluding estimated of diversity from distressed marginal populations of *R. draytonii* in southern California, these same signals of northward range expansion were mirrored in allelic richness A_R, observed heterozygosity H_O, and gene diversity H_S, where sequential groups of samples from north to south show higher values (Table 2). The pattern was reversed for relatedness, as expected, indicating that pairwise relatedness of frogs in the same ponds is higher in the northern part of the range than the south.

Mitochondrial diversity was also consistent with a northward range expansion, as evidenced by longer branch lengths (Fig. 4) and greater numbers of haplotypes p, nucleotide differences k, and nucleotide diversity π among the southern samples. This pattern however, depends on the inclusion of historical samples from the Santa Rosa Plateau (Riverside Co., California) and Baja

California; without these samples, diversity estimates for southern California samples was on par with their northern counterparts in the Sierra Nevada and the Bay Area. Because of the recent disappearance of *R. draytonii* throughout much of southern California and the lack of genetic samples for comparison, it is difficult to tell how much genetic diversity was lost as a consequence of local extirpation over the past 50+ years. We suspect that it was substantial, given that historical samples from the Santa Rosa Plateau and the Sierra San Pedro Martir share a common haplotype that is notably divergent from all others sampled throughout the range to date; thus historically intervening populations between these locations must have contained at least this same unique variation, and probably more that has gone undetected.

In addition to the effects of northward range expansion, at least some of the lack of variability and strong differentiation among Sierra Nevada populations is likely due to genetic drift, especially because gene exchange is highly restricted among these populations. If demographic declines have occurred in these ponds and their isolation restricts novel alleles from entering through gene flow, then the purging of variation through genetic drift may be prevalent. In fact, drift is expected to eliminate variability more quickly in neutral loci with low polymorphism to begin with – consistent with this prediction, we found that the two most invariant loci in this study were among the least polymorphic in the southern samples from Richmond et al. (2013). Our tests of IBD are also consistent with the effects of drift, given that the IBD signal disappeared once we accounted for spatial structuring in the data (i.e. Sierra Nevada vs. Bay Area). If drift is a factor, the random fixation or elimination of different alleles across populations could erase the signals of IBD in the absence of gene flow. This process would be accelerated if population sizes have been substantially reduced over time.

4.4. What management actions should be taken?

Although there is no evidence of contemporary gene flow between any of the Sierra Nevada *R. draytonii* populations, it is likely that they were once part of larger metapopulations within their respective watersheds. This assumption is based on population dynamics in other parts of the species range (Bulger et al., 2003; Fellers and Kleeman, 2007; Tatarian, 2008; Richmond et al., 2013), information about the historical distribution in the Sierra Nevada (Barry and Fellers, 2013), the former interconnection of populations as indicated by shared mtDNA haplotypes across ponds (and microsatellite alleles in several frogs at Bear Creek), and the presence of metapopulation structuring at the watershed level in other western North American amphibians (Shaffer et al., 2000; Monsen and Blouin, 2003; Funk et al., 2005; Lind et al., 2011). Although some ponds share identical mtDNA haplotypes, patterns of variation in the microsatellite alleles suggest that drift has caused substantial genetic divergence, and the absence of current gene flow prevents admixture of different alleles across ponds. Shared mitochondrial haplotypes simply reflects retained ancestral polymorphism, as the amount of time since isolation of these populations is insufficient for mutation to have caused those haplotypes to diverge. As such, we consider each Sierran *R. draytonii* population to be a genetically distinctive unit.

While the low genetic diversity and small N_e for Sierran *R. draytonii* presents some cause for concern, unless there is evidence of adverse fitness effects as a consequence of this level of diversity, we suggest that the most prudent management efforts at this juncture should focus on increasing habitat quality, quantity and occupancy. These objectives can be achieved by creating additional breeding ponds and managing existing ponds to support breeding within dispersal distance of occupied habitat (approximately 0.2 – 3.2 km; Bulger et al., 2003; Fellers and Kleeman, 2007). Pond management efforts may include: (1) inundation periods that support metamorphosis; (2) a mix of deep (>1.0 m) water cover and warmer shallow areas for tadpole development; (3) surface water temperatures above 10 °C; (4) a mix of open surface water and vegetative cover; and (5) removing nonnative fish, crayfish and bullfrogs (Ford et al., 2013). We favor these tactics over translocation at this time because of the genetic uniqueness of each of these populations, and to avoid any potentially undesirable effects of introducing non-local genetic variation (see Hoffman and Blouin, 2004 for additional discussion). This approach would also preserve the historical phylogeographic structure of the species in the Sierra Nevada, and therefore the natural processes that have generated, distributed and maintained that diversity. Pond size is also known to influence N_e in other amphibians (Wang, 2010), therefore increasing habitat availability may also be a way to increase the N_e of very small populations such as Hughes Pond, and could potentially restore more of the historical, metapopulation-like structure to targeted locations.

Finally, we cannot dismiss the possibility that small N_e and low levels of gene flow are natural characteristics of *R. draytonii* in some or all parts of its range (see Barry and Fellers (2013) for further discussion), in which case genetic diversity may have been declining over many generations. Other western North American ranid frog species also have similarly small contemporary effective sizes, genetic diversity varies substantially among species, and high population differentiation appears to be the norm (Phillipsen et al., 2011). Whether our findings for Sierran *R. draytonii* are due to natural demographic effects or recent anthropogenic disturbance is key to determining how best to manage these populations.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2014.02.026.

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