

Evidence for concerted movement of nuclear and mitochondrial clines in a lizard hybrid zone

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Abstract

Moving hybrid zones provide compelling examples of evolution in action, yet long-term studies that test the assumptions of hybrid zone stability are rare. Using replicated transect samples collected over a 10-year interval from 2002 to 2012, we find evidence for concerted movement of genetic clines in a plateau fence lizard hybrid zone (*Sceloporus tristichus*) in Arizona. Cline-fitting analyses of SNP and mtDNA data both provide evidence that the hybrid zone shifted northward by approximately 2 km during the 10-year interval. For each sampling period, the mtDNA cline centre is displaced from the SNP cline centre and maintaining an introgression distance of approximately 3 km. The northward expansion of juniper trees into the Little Colorado River Basin in the early 1900s provides a plausible mechanism for hybrid zone formation and movement, and a broadscale quantification of recent land cover change provides support for increased woody species encroachment at the southern end of the hybrid zone. However, population processes can also contribute to hybrid zone movement, and the current stability of the ecotone habitats in the centre of the hybrid zone suggests that movement could decelerate in the future.

Keywords: hybridization, introgression, RADseq, *Sceloporus*, SNP

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Introduction

Hybrid zones are regions where genetically distinct populations meet and produce hybrids, and therefore provide natural settings for the study of speciation (Barton & Hewitt 1985; Harrison 1993). Moving hybrid zones provide compelling examples of evolution in action as a consequence of environmental changes on species distributions (Taylor *et al.* 2014). Anthropogenically mediated habitat modification and climate change are two environmental factors reported to have a direct effect on hybrid zone movement (Buggs 2007; Chunco 2014, Taylor *et al.*, 2015).

The formation of hybrid zones and their associated character clines can result from secondary contact of populations that diverged in geographic isolation, or divergent selection across an environmental gradient

(Barton & Hewitt 1985; Harrison 1993). Traits that are fixed in parental populations often exhibit continuous geographic change in frequency across a hybrid zone (Endler 1977; Harrison 1990), and endogenous (genomic) and exogenous (environmental) selection on the admixed hybrid offspring determines the location, size and potential for movement of a hybrid zone. Accordingly, clines across hybrid zones can be characterized by their centre, width and shape, and clines across different characters are expected to be coincident (share the same centre) and concordant (equivalent width and shape) when selection is uniform and the environment is stable. However, hybrid zones are expected to move when selection or migration into the hybrid zone is asymmetric, or if the environment changes.

Studies of hybrid zone movement are rare because they typically require long-term replicated transect sampling to track the position of the hybrid zone at different times (Buggs 2007). Alternatively, patterns of asymmetric introgression among loci from samples

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collected at a single point in time can provide evidence for hybrid zone movement, because neutral markers are expected to lag behind non-neutral loci to produce a pattern of unidirectional introgression in a moving hybrid zone (Moran 1981; Rohwer *et al.* 2001; McGuire *et al.* 2007). However, even in a stationary hybrid zone, asymmetric patterns of introgression can also result from neutral diffusion between populations, or differential selection pressure across traits of an organism, for example between the nuclear genome and cytoplasmic DNA markers (Dasmahapatra *et al.* 2002). Replicated transect sampling makes it possible to distinguish between differential selection and hybrid zone movement. Cline centre differences derived from replicated sampling provide direct evidence of movement, whereas differences from a single point in time could be reflective of differential selection. As a consequence, any noncoincident cline centres observed at one time point should shift in unison through time in a moving hybrid zone.

Here, we use replicated transect samples collected over a 10-year interval (2002 and 2012) to test for concerted movement across nuclear and mitochondrial markers in a hybrid zone between grassland and juniper populations of plateau fence lizards (*Sceloporus tristichus*) in Arizona (USA). The hybrid zone is located in eastern Arizona in the Little Colorado River Basin along Silver Creek, primarily between the cities of Show Low and Holbrook (Fig. 1a). Across the hybrid zone, the vegetation transitions from Great Basin Conifer Woodland in the south to Great Basin Grassland in the north. Hybrid lizards are found in the ecotone habitats at the

interface of the vegetation transition between juniper and grassland habitats (Leaché & Cole 2007). The conversion of grasslands into woodlands containing juniper (*Juniperus*) and pinyon pine (*Pinus*) is considered one of the most pronounced vegetation changes to have occurred in the western United States in the last century (Miller & Tausch 2001). Historically, grassland environments predominated in the region, which would have facilitated a broader distribution of the grassland lizard population. In addition, beginning in the 1890s, extensive cattle overgrazing throughout the hybrid zone converted grassland habitats into shrublands dominated by juniper trees (Archer 1994; Abruzzi 1995), which in this landscape would have caused a northward expansion of shrublands during the 20th century. We investigate recent changes in land cover to understand the ecological context of hybrid zone movement. These changes provide insight into the origin, maintenance and perhaps future direction and rate of shifts in the hybrid zone.

The parental populations found at opposite ends of the hybrid zone differ with respect to morphology, ecology, chromosome polymorphisms and nuclear and mitochondrial DNA (mtDNA) divergence (Leaché & Cole 2007; Leaché 2011). The populations at opposite ends of the hybrid zone were once considered different subspecies based on extensive morphological differences in body size, scale counts, colour and colour pattern (Smith *et al.* 1992). The chromosomal polymorphism found in the hybrid zone includes three distinct pericentric inversions on chromosome 7, and hybrids have

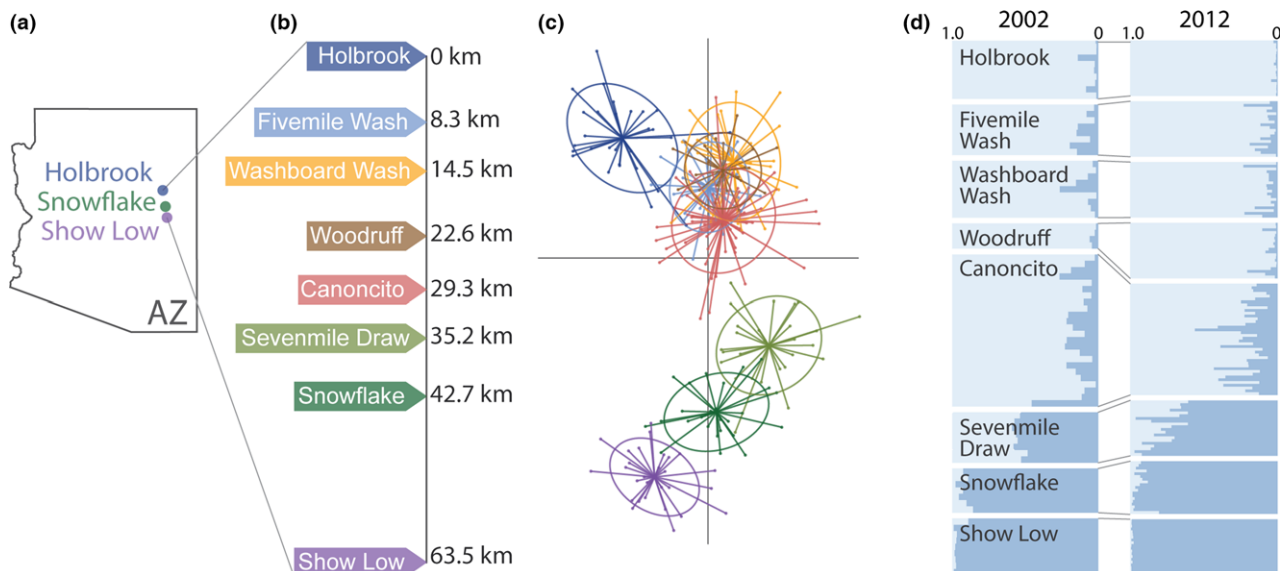


Fig. 1 Overview of SNP variation in the *Sceloporus tristichus* hybrid zone in Arizona. (a) The hybrid zone is located in Arizona between the cities of Holbrook and Show Low. (b) The sampling transect includes eight sites spanning 63.5 km. (c) DAPC clustering using the combined 2002 and 2012 SNP data. (d) STRUCTURE plots ($K = 2$) for the 2002 and 2012 SNP data. [Colour figure can be viewed at wileyonlinelibrary.com]

heteromorphic pairs of chromosome 7 inversions (Cole 1972; Leaché & Cole 2007). The genetic diversity in the hybrid zone is high, with a maximum sequence divergence exceeding 10% for mtDNA (uncorrected pairwise divergence; Leaché & Cole 2007) and F_{st} values as high as 0.6 at anonymous nuclear loci (Leaché 2011). Population divergence genetic analyses using multiple loci (Leaché 2011) suggest that the populations diverged during the Pleistocene (45 000–1.9 ma) and that migration rates between the populations are high (2.2–2.5 migrants per generation).

Comparisons of cline centres using chromosome polymorphism data collected in the 1970s and 2002 suggested that that hybrid zone is shifting northwards by approximately 0.5 km per decade (Leaché & Cole 2007). The inferred direction of cline movement is consistent with the hypothesis that the southern lizard population moved northwards along with the juniper trees that invaded habitats previously dominated by grasslands. In this study, we test the northward movement hypothesis using single nucleotide polymorphism (SNP) and mtDNA data using replicated transect sampling conducted in 2002 and 2012. We compare cline centre estimates between sampling periods to test for hybrid zone movement. For clines with different centres, we might expect to observe concerted cline movement between time periods that result in clines that move in a lockstep formation. This synchronous movement of clines could be difficult to detect if the demographics of the populations changed through time, as mtDNA and nuclear loci will respond differently to demographic shifts, and such shifts can influence the properties of clines.

Materials and methods

Sampling

We collected 179 samples from eight localities spanning the hybrid zone in 2012 (Table 1; Fig. 1). We compared

the 2012 samples to 95 samples collected from the same localities in 2002 (Leaché & Cole 2007; Leaché 2011). The sampling transect extends 63.5 km from grasslands (north) to juniper woodlands (south), and lizards with intermediate phenotypes and genotypes inhabit canyon ecotones connecting these habitats (Leaché & Cole 2007; Leaché 2011). Lizard collecting was approved by the State of Arizona Game and Fish Department (SP #568189). Animal research was approved by the University of Washington Office of Animal Welfare (IACUC #4209-01).

SNP data collection

Genomic DNA was isolated from fresh liver samples using QIAGEN DNEASY extraction kits (QIAGEN Inc.). We collected SNP data using the double-digestion restriction site-associated DNA sequencing (ddRADseq) protocol (Peterson *et al.* 2012). We double-digested 500 nanograms of genomic DNA for each sample with 20 units each of a rare cutter *SbfI* (restriction site 5'-CCTGCAGG-3') and a common cutter *MspI* (restriction site 5'-CCGG-3') in a single reaction with the manufacturer-recommended buffer (New England Biolabs) for 8 h at 37 °C. Fragments were purified with Agencourt AMPure beads before ligation of barcoded Illumina adaptors onto the fragments. Custom oligonucleotide sequences were used for barcoding and adding Illumina indexes during library preparation (Peterson *et al.* 2012). The combination of eight unique barcodes and 18 unique indexes allowed us to pool samples in sets of eight, and to multiplex 18 pools (=144 samples) on one sequencing lane. The libraries were size-selected (between 415 and 515 bp after accounting for adapter length) on a Blue Pippin Prep size fractionator (Sage Science). The final library amplification used Illumina index primers and high-fidelity *Taq* polymerase (New England Biolabs). The fragment size distribution and concentration of each pool were determined on an

Table 1 Hybrid zone transect sites (and GPS coordinates) compared over a 10-year interval using replicate samples collected in 2002 and 2012. Sample sizes for mtDNA and SNPs are shown for each year

Site name	Latitude (°N)	Longitude (°W)	2002		2012	
			mtDNA	SNPs	mtDNA	SNPs
Holbrook	34.90371	110.18208	13	9	20	20
Fivemile Wash	34.83582	110.1449	8	8	9	20
Washboard Wash	34.79292	110.09873	11	9	18	20
Woodruff	34.73801	110.03783	9	4	12	20
Canoncito	34.6678	110.0377	25	24	18	40
Sevenmile Draw	34.6025	110.0624	12	8	10	20
Snowflake	34.52789	110.08072	5	7	14	19
Show Low	34.33398	110.11372	12	9	20	20

Agilent 2200 TapeStation, and qPCR was performed to determine sequenceable library concentrations before multiplexing and sequencing on two separate instruments, one Illumina HiSeq 4000 lane (50 bp, single-end reads; 142 samples) at the QB3 facility at UC Berkeley, and two Illumina HiSeq 2500 lanes (50 bp, single-end reads; 144 samples) at the DNA Sequencing Center, Brigham Young University.

Bioinformatics

We processed raw Illumina reads using the program PYRAD version 3.0.63 (Eaton 2014). We demultiplexed samples using their unique barcode and adapter sequences, and sites with Phred quality scores under 99% (Phred score = 20) were changed into 'N' characters, and reads with $\geq 10\%$ N's were discarded. Each read was reduced to 39 bp after the removal of the 6 bp restriction site overhang and the 5 bp barcode. The filtered reads for each sample were clustered using the program VSEARCH version 1.1.0 (Rognes *et al.* 2016) and then aligned with MUSCLE (Edgar 2004). This clustering step establishes homology among reads for each sample. We assembled the ddRADseq data using a clustering threshold of 92%. The clustering threshold is an important parameter that affects the number loci, amount of variation, and in some cases the inferences from population genetic (Harvey *et al.* 2015) and phylogenetic analysis (Leaché *et al.* 2015). We selected a relatively stringent clustering threshold of 92% to reduce the risk of combining paralogs, while still accommodating a realistic level of sequence variation (up to three variable sites per RAD locus) observed in anonymous nuclear loci used in a previous analysis of the hybrid zone (Leaché 2011). As an additional filtering step, consensus sequences were discarded that had low coverage (<10 reads), excessive undetermined or heterozygous sites (>4) or too many haplotypes (>2 for diploids).

The consensus sequences were clustered across samples using the same threshold used to cluster data within species (92%). This step establishes locus homology among samples. Each locus was aligned with MUSCLE, and a paralog filter that removes loci with excessive shared heterozygosity among samples was applied (paralog filter = 200). The final data set only included RAD loci with no more than 8.5% missing data. Allowing higher levels of missing data will increase the number of SNPs in the final data matrix, but this relatively stringent threshold was chosen to reduce any negative influences of missing data on downstream population genetic and cline-fitting analyses. Only one random SNP was sampled from each putatively unlinked ddRAD locus for the final data matrix. Demultiplexed sequences are available on the NCBI Sequence Read Archive

(SRP093397), and the final assemblies are available on Dryad (doi:10.5061/dryad.19gp1).

Population structure

We determined whether the SNP data could resolve population structure in the hybrid zone and discriminate between the sampling locations using discriminant analysis of principal components (DAPC; Jombart *et al.* 2010) in the R package ADEGENET (Jombart & Ahmed 2011). We analysed all samples from 2002 and 2012 both separately and together. We ran DAPC clustering using 85 PCAs and subsequently optimized the number of PCAs to 14 using the *optim.a.score* function. We examined temporal changes in population admixture using STRUCTURE version 2.3.1 (Pritchard *et al.* 2000; Falush *et al.* 2007). Previous population genetic analyses of the hybrid zone using multilocus nuclear data found that the number of populations (K) is 2 (Leaché 2011), and this is the intuitive model for a hybrid zone. We estimated the optimal value of K for the SNP data by evaluating K values from 1 to 4 (10 000 burn-in, 100 000 data collection, four replicates each) using STRUCTURE HARVESTER version 0.6.94 (Earl & vonHoldt 2012) and the Evanno method (Evanno *et al.* 2005). Final analyses with the optimal K value ($K = 2$) were repeated twice with a burn-in period of 100 000 cycles, and 1 000 000 additional cycles. Analyses were performed for each time period separately using an admixture model with correlated allele frequencies. For each sampling site, we estimated the average admixture proportion (Q), which represents the fraction of membership of each locality to the northern and southern populations. Estimates of Q were averaged across the two replicate runs, and these average values were used in subsequent maximum-likelihood (ML) cline-fitting analyses.

Mitochondrial DNA analysis

The *Sceloporus tristichus* hybrid zone in Arizona involves two distinct populations, yet previous studies have found foreign mtDNA haplotypes belonging to different populations and species in the hybrid zone at a high frequency (Leaché & Cole 2007; Leaché 2011). We amplified and sequenced the entire mitochondrial *ND1* protein-coding gene (969 bp) for the 2012 samples using standard methods (Leaché & Cole 2007). The *ND1* sequences collected for the 2012 samples were aligned (the length of the *ND1* protein-coding gene is conserved in the hybrid zone sample) with an existing data matrix containing the 2002 samples and other *S. tristichus* and *S. cowlesi* populations from the southwestern United States (Leaché & Cole 2007) for a final data matrix of 540 samples. New mtDNA sequences are deposited in

GenBank (Accession nos KY192531–KY192709), and the alignment is available on Dryad (doi:10.5061/dryad.19gp1).

We estimated a ML phylogeny of the *ND1* gene using RAXML version 8.2 (Stamatakis 2014). We used the GTRGAMMA substitution model and bootstrapping with 1000 replicates. The GTR model is appropriate for these samples, as shown in previous analyses of this hybrid zone (Leaché & Cole 2007). We assigned the mtDNA haplotype obtained for each individual into one of four clades: (i) *S. tristichus* north represents the northern hybrid zone population; (ii) *S. tristichus* south contains the southern hybrid zone population; (iii) *S. tristichus* west contains western populations that leak into the hybrid zone; and (iv) *S. cowlesi* is ~10% divergent from *S. tristichus*. Haplotypes from *S. cowlesi* leak into the hybrid zone from the east, possibly following the Little Colorado River. Assigning haplotypes into each of these four clades using the ML phylogeny was necessary to determine the frequency of northern and southern haplotypes at each locality after subtracting the haplotypes belonging to the western clade of *S. tristichus* and to *S. cowlesi*. For the 2002 mtDNA haplotype assignments and frequencies, we used previously published data (Leaché & Cole 2007). We also calculated the frequency of foreign mtDNA haplotypes at each locality for the 2002 and 2012 samples to examine changes in the distribution of mtDNA haplotypes across the hybrid zone through time.

Cline-fitting analyses

We performed ML cline-fitting analyses of the SNP and mtDNA data for 2002 and 2012 using the R package HZAR (Derryberry *et al.* 2014). Clines were fit with a null model and three different models that varied the assumptions regarding cline parameters; model 1 assumed trait intervals fixed at 0 and 1 without tail fitting, model 2 used estimated trait intervals without tail fitting and model 3 estimated trait intervals and tail fitting (Szymura & Barton 1986; Szymura 1991). We performed model selection using AICc scores to determine the preferred model and then extracted the ML model parameters. We estimated separate clines for SNPs and mtDNA and for different sampling periods, 2002 and 2012. In addition, to verify that differences in clines were not due to sample size differences, we conducted 50 random replicate analyses of the 2012 SNPs using a reduced sampling scheme aimed at matching the number of individuals included from each locality to that of our 2002 sampling. Finally, we tested for significant differences between clines by conducting constrained HZAR analyses using cline centres constrained to each data set. Comparisons between constrained and free models

with AICc scores differing by >2 were considered significantly different.

Land cover and environmental analyses

We examined the ecological context of hybrid zone movement by measuring the extent of woody plant encroachment using 30-m-resolution NATIONAL LAND COVER DATABASE maps (Jin *et al.* 2013) produced using circa 2001 and circa 2011 landsat imagery composites. Net woody encroachment was assessed at 1 km grid resolution by taking the sum of the total area of transitions from less woody (grass, pasture, shrub) to more woody (shrub, forest) land cover types minus the sum of the total area of transitions from nonwoody to woody land cover types. Because the land cover classes are relatively coarse, and may therefore underestimate changes in vegetation density, we also measured changes in juniper shrub density within 1 km of the collection locations by manually digitizing shrub locations on 1996 and 2011 aerial photographs accessible in Google Earth.

We also investigated climate trends in the hybrid zone by comparing environmental variables for Navajo County, Arizona (which encompasses the study area) over two time periods. We quantified both short-term (30 years; 1979–2009) and long-term (60 years; 1949–2009) climate trends using data from NOAA CLIMATE EXPLORER version 2, which were based on weather station records interpolated with the method of Maurer *et al.* (2002). The climate trends measured included the linear rate of change in mean daily maximum temperature (°C/decade), mean daily minimum temperature (°C/decade) and mean daily precipitation (mm/day/decade). These variables are sufficient for summarizing trends in warming and drying across the hybrid zone, which could influence the distribution of grassland and woodland habitats.

Results

SNP data

A summary of the final SNP data is presented in Table S1 (Supporting information). The average number of loci recovered ranged from 970 to 1041 (per individual); the average depth ranged from 16.4 to 36.2. The final data set contained 745 SNPs with no more than 8.5% missing data.

Population structure

Population structure estimated using DAPC for all samples from 2002 and 2012 combined (257 total) shows

clear separation between locations at opposite ends of the hybrid zone (Fig. 1c). Four sample sites at the northern end of the hybrid zone (Fivemile Wash, Washboard Wash, Woodruff and Canoncito) are indistinguishable in the DAPC plot, while four other locations are distinguishable, including Holbrook, Sevenmile Draw, Snowflake and Show Low (Fig. 1c). Separate DAPC plots for 2002 and 2012 are provided in Fig. S1 (Supporting information).

The Evanno method selected $K = 2$ as the best-fit model for the number of populations in the hybrid zone using SNP data (Table S2, Supporting information), suggesting that two populations are arranged from north to south with a narrow zone of admixture (Fig. 1d). Structure analyses performed with $K = 2$ for the 2002 and 2012 samples produced Q values ranging from 0.897 to 0.987 for the northern hybrid zone samples from Holbrook to Woodruff (Table S3, Supporting information; Fig. 1d). Variation in the estimated Q values between replicate runs was <0.001 . The southern hybrid zone samples (Snowflake and Show Low) had Q values between 0.013 and 0.081. The two locations near the centre of the hybrid zone had Q values between 0.231 (Sevenmile Draw) and 0.857 (Canoncito).

Mitochondrial DNA analysis

The ML phylogenetic analysis provides strong support (bootstrap $>70\%$) for the northern and southern hybrid zone clades (Fig. S2, Supporting information). Northern mtDNA haplotypes are found in high frequency (>0.8) from Holbrook to Sevenmile Draw (Table S3, Supporting information). Southern mtDNA haplotypes are fixed in the two southernmost sites, Snowflake and Show Low (Table S3, Supporting information). Haplotypes belonging to *Sceloporus cowlesi* are found in the middle of the hybrid zone from Fivemile Wash in the north to Sevenmile Draw in the south (Table S4, Supporting information). Haplotypes belonging to a western population of *S. tristichus* are found in low frequency in Sevenmile Draw (0.15) and Snowflake (0.26) (Table S4, Supporting information).

Cline-fitting analysis

Cline-fitting analyses conducted under four different models compared using AICc scores support model 1 (trait intervals fixed at 0 and 1, without tail fitting) for the SNP and mtDNA data for each sampling period (Table S5, Supporting information). Cline-fitting results for the SNP and mtDNA data support hybrid zone movement between 2002 and 2012 (Table 2; Fig. 2). The SNP cline centre shifted to the north by approximately 1.93 km between 2002 and 2012 (2 log-likelihood unit support limits, 2LL = 0.87–4.23 km; Table 2). The mtDNA cline supports a similarly spaced shift of 2.24 km (2LL = 1.8–5.21 km; Table 2). The cline centres for the SNPs and mtDNA are offset from one another in each time period, and the mtDNA cline is shifted to the south by approximately 3 km in both 2002 (ML = 3.04 km; 2LL = 1.31–5.35 km) and 2012 (ML = 2.73 km; 2LL = 3.98–4.39 km). The SNP cline centre models are significantly different between 2002 and 2012. Constraining the mtDNA data (2002 or 2012) to fit the 2012 SNP cline centre resulted in significantly worse fits (Table 3). However, constraining the 2012 mtDNA data to the 2002 SNP model did not produce a significant AICc difference, suggesting that a decade of hybrid zone movement shifted the mtDNA cline centre into alignment with the past location of SNP cline centre (Table 3). The mtDNA clines, although displaced from one another to the same degree as the SNP data, are not significantly different (Table 3). Differences in sample sizes between the 2002 and 2012 data are not sufficient to explain the observed differences in cline centres; cline centre estimation of the subsampled data differs by only 0.13 km (SD = 0.54) from the full data set (Table S6; Fig. S3, Supporting information).

Land cover and environmental analyses

Recent land cover changes support a net increase of woody species encroachment of approximately 25% in the south of the hybrid zone near Show Low (Fig. 3). Land cover turnover is minimal throughout the remainder of the hybrid zone, and in some locations, the area

Table 2 Evidence for concerted movement of genetic clines in the *Sceloporus tristichus* hybrid zone. Temporal comparisons of ML cline centres (kilometres from Holbrook) using SNPs and mtDNA support an approximate 2 km northward shift. For each sampling period, the mtDNA cline is south of the SNP cline and maintaining an introgression distance of approximately 3 km. The 2LL support limits are in parentheses (minimum, maximum). The shift is significant for SNPs, but not mtDNA (see Table 3)

	2002	2012	Northward shift
SNPs	34.71 (32.08, 38.97)	32.78 (31.21, 34.74)	+1.93 (+0.87, +4.23)
mtDNA	37.75 (33.39, 44.34)	35.51 (35.19, 39.13)	+2.24 (+1.8, +5.21)
Introgression distance	-3.04 (-1.31, -5.37)	-2.73 (-3.98, -4.39)	

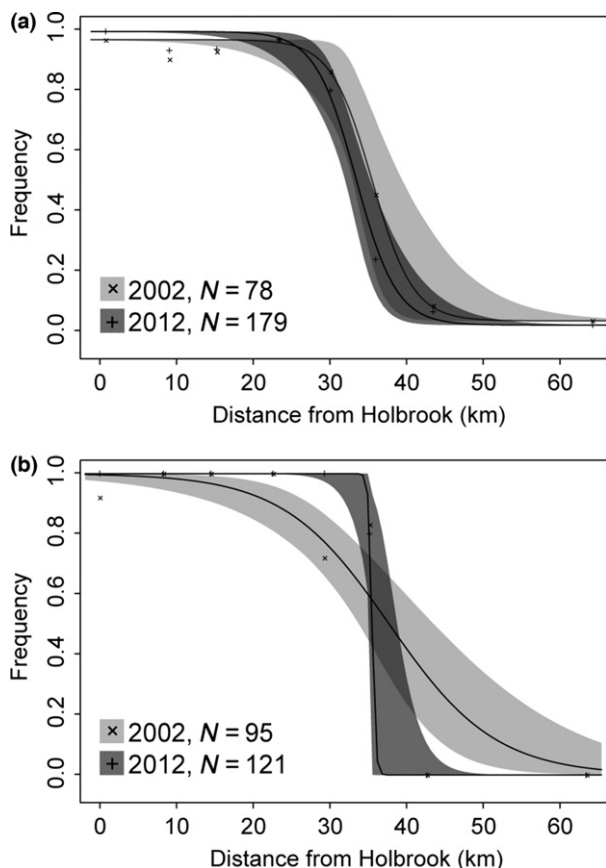


Fig. 2 Temporal comparisons of clines, (a) SNPs, (b) mtDNA, in the *Sceloporus tristichus* hybrid zone. ML cline centre line is in black, confidence intervals are shaded and observed admixture proportions are shown with 'x' symbols for 2002 and '+' symbols for 2012.

Table 3 AICc scores comparing the best-fit unconstrained cline centre for each data set (first column) to constrained models enforcing the alternative ML cline centres. The best model for each data set is highlighted in bold. AICc score differences greater than 2 points are considered significantly different and highlighted with an asterisk (*)

Data set	Cline centre model			
	mtDNA 2002	mtDNA 2012	SNPs 2002	SNPs 2012
mtDNA 2002	17.87	3.86	6.11*	25.12*
mtDNA 2012	18.57	2.67	4.03	17.09*
SNPs 2002	19.62	9.74*	3.66	13.05*
SNPs 2012	23.57*	24.53*	5.99*	8.65

of shrublands has decreased slightly (Fig. 3). A comparison of aerial photographs from the centre of the hybrid zone taken in 1996 and 2012 shows no net change in juniper cover, supporting stable ecotone habitats in the

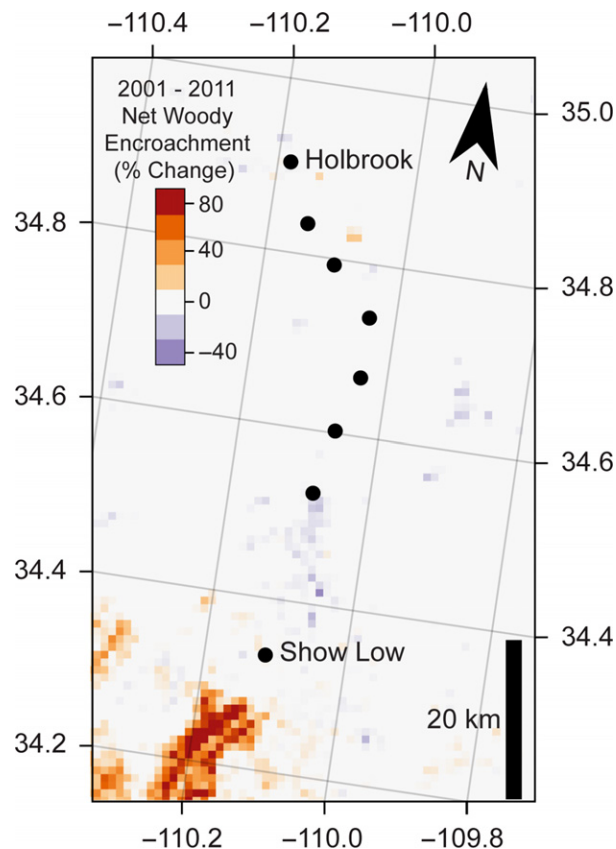


Fig. 3 Land cover change analysis shows a net increase of woody species in the southern end of the hybrid zone. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 4 Climate trends in the hybrid zone over the short term (1979–2009) and long term (1949–2009). Mean daily maximum and minimum temperatures have both increased, and the region has become drier (decrease in the mean daily precipitation) over the short term

Climate variable	Short-term trend	Long-term trend
Mean daily maximum temperature (°C/decade)	+0.357	+0.077
Mean daily minimum temperature (°C/decade)	+0.255	+0.387
Mean daily precipitation (mm/day/decade)	-0.106	+0.01

immediate vicinity of the collection locations (Fig. S4, Supporting information). An analysis of climatic trends suggests that the hybrid zone has experienced significant warming and drying over the last 30 years (Table 4). Long term trends are more equivocal; the mean daily maximum temperature increase is <0.1 °C, and precipitation shows a minor increase of 0.01 mm/day/decade from 1949 to 2009.

Discussion

We provide evidence for a hybrid zone that moved over a 10-year period. We detected this movement using markers from both the nuclear and mitochondrial genomes (Table 2; Fig. 2). The nuclear and mtDNA cline centres appear to have shifted in synchrony over this decadal period despite the displacement of their cline centres. We relied on replicated transect sampling to detect cline movement over this decadal time interval. Replicated transect sampling has a number of advantages compared to studying cline movement with samples from a single time period, including the ability to directly quantify changes in population allele frequencies through time, and to detect changing patterns of introgression among loci. Hybrid zones are important systems for studying speciation and evolution, and replicated sampling over time remains the most reliable method for detecting and studying their movement (Buggs 2007).

The shift in cline centres measured between 2002 and 2012 is significant for the SNP data; however, the mtDNA shift is not significant (Table 3). This lack of significance for mtDNA cline movement is likely due to the increased cline width estimated for 2002 (27.7 km) vs. 2012 (0.9 km). The source of this discrepancy is one aberrant mtDNA haplotype frequency for Canoncito in 2002 (Fig. 2; Table S3, Supporting information), and this outlier causes the cline to deviate from the characteristic pattern of gradual change in frequency across the transect (Fig. 2). Despite the large difference between mtDNA cline widths, the mtDNA cline centre is estimated to be approximately 3 km south of the SNP cline centre in both 2002 and 2012 (Table 2), which is consistent with the prediction of cline movement.

An important factor to consider in this hybrid zone is the influx of mtDNA haplotypes that are more closely related to other populations of *Sceloporus tristichus* and from another species, *S. cowlesi* (Table S4; Fig. S2, Supporting information). The preponderance of these foreign mtDNA haplotypes, and their changing frequencies through time (Table S4, Supporting information), could be responsible for the disparity in mtDNA cline widths measured between 2002 and 2012 (Fig. 2; Table S6, Supporting information). It is possible that change in mtDNA haplotype composition of populations observed between 2002 and 2012 is the result of demographic shifts, which can affect cline width (Slatkin & Maruyama 1975; Polechová & Barton 2011).

We measured an introgression distance of approximately 3 km that causes the mitochondrial cline to be shifted to the south of the nuclear cline, and this introgression distance is maintained between 2002 and 2012 (Table 2; Fig. 2). The average home range size for male *Sceloporus tristichus* is approximately 1 km², and females

have even greater dispersal limitations (Ferner 1974; Haenel *et al.* 2003). The unequal dispersal capabilities of males and females combined with the selectively neutral nature of mtDNA increase the likelihood of observing discordant mtDNA and nuclear clines. Inferred differences in cline centres between data types may be due to the geographic movement of populations, or because of differential selection on these distinct characters (Brumfield *et al.* 2001). Clines inferred from nuclear and mtDNA are not unexpected to be discordant, as inheritance patterns and selection are distinct for these two genomes (Ballard & Whitlock 2004). Introgression can be caused by a geographic shift in a hybrid zone, and observing a spatial lag in cline centres is common with mtDNA (Secondi *et al.* 2006; McGuire *et al.* 2007). Sex-biased life history differences, specifically dispersal distances, can also generate differences in the zones of intergradation between the nuclear and mtDNA genomes (Funk & Omland 2003). Similarly, nonrandom, preferential interspecific mating can lead to a lack of coincidence in nuclear and mtDNA cline centres.

Although once thought to be rare, empirical cases documenting the geographic movement of hybrid zones are coming to light across a variety of taxa (e.g. Carling & Zuckerberg 2011; Smith *et al.* 2013). The rate of movement for this hybrid zone is approximately 2 km/decade, which is low compared to the mean rate of approximately 10 km/decade found in other hybrid zones (Buggs 2007; Taylor *et al.* 2014). However, species-specific dispersal rates have a large impact on the rate of hybrid zone movement, and *Sceloporus* lizards have limited dispersal capabilities compared to the species studied in other moving hybrid zones (e.g. birds and butterflies; Buggs 2007). The 2 km/decade rate for the *S. tristichus* hybrid zone estimated here with SNPs and mtDNA is faster compared to the 0.5 km/decade estimate obtained using chromosome polymorphism data comparisons between the 1970s and 2002 (Leaché & Cole 2007); however, the chromosome estimate was based on smaller sample sizes and nonoverlapping population samples.

Why is the hybrid zone moving? Hybrid zones move due to intrinsic factors such as population density differences and interspecific interactions (Engler *et al.* 2013), asymmetric hybridization rates (Endler 1977; Barton 1979), dominance drive (Mallet 1986), or extrinsic factors such as environmental (Hairston *et al.* 1992) or climate change (Walls 2009; Taylor *et al.* 2015). However, these processes are not mutually exclusive, and it is difficult to directly test all of the alternative explanations for hybrid zone movement simultaneously. The specialized ecologies of the grassland and juniper populations of *Sceloporus tristichus* make them susceptible to habitat shifts, and the hybrid zone may have formed

during the dramatic environmental alterations that affected these habitats near the turn of the 20th century (Archer 1994; Abruzzi 1995). However, habitat changes appear to have been minimal during the 2000s, with the exception of extensive encroachment of woody species at the southern end of the hybrid zone (Fig. 3). Temporal changes in the relative densities of populations are implicated in the hybridization between *S. undulatus* and *S. woodi* in Florida, where forest clear-cuts likely caused dramatic population density differences (Robbins *et al.* 2014). Population densities of *S. tristichus* at the southern end of the hybrid zone likely increased in response to the encroachment of woody vegetation in this region.

We suspect that the hybrid zone movement detected from 2002 to 2012 is a result of an increased population density of juniper lizards in the southern portion of the hybrid zone. A study of population density differences between *S. tristichus* populations in central Arizona found that their abundance was significantly higher in forest vs. chaparral habitats (Cunningham *et al.* 2002). Within forest habitats, *S. tristichus* is significantly more likely to be found associated with dead pinyon pine and juniper trees than living trees, suggesting that dead vegetation is an important component of their microhabitat (James & M'Closkey 2003). Their preference for dead trees could be a reflection of adaptation to disturbed habitats; for example, the relative abundance of *S. tristichus* was seven times greater in burned than unburned forest (Cunningham *et al.* 2002).

What does the future hold for this hybrid zone? If environmental conditions and population densities were to stabilize, then we predict that the rate of movement of nuclear and mitochondrial clines will decelerate. This outcome seems unlikely. Environmental changes occurring at broad and local scales will combine to shape the future of the populations interacting in the hybrid zone. For example, drought conditions and higher temperatures have caused die-offs of woodlands across the southwest, which will have consequences for the distributions and population abundances of species adapted to this ecosystem (Breshears *et al.* 2005; Floyd *et al.* 2009). Other ecological factors, such as the fire regime and anthropogenic habitat modifications, also contribute towards shaping the availability of microhabitats and the local abundance of populations. Unequal population densities between grassland and woodland populations of *S. tristichus* are likely to maintain a pattern of discordant introgression and continued hybrid zone movement.

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A.D.L. designed the study and analysed data; A.D.L. and J.A.G. conducted fieldwork in 2012; J.A.G. collected ddRADseq data; R.B.H. conducted cline analyses; and I.B. conducted land cover analyses. All authors contributed to the text and approved the final manuscript.

Data accessibility

Mitochondrial DNA sequences: GenBank Accession nos KY192531–KY192709; ddRADseq data: NCBI SRA Accession no. PRJNA350173 (SAMN05936460–05936716). Final data assemblies are available on the Dryad Digital Repository: doi:10.5061/dryad.19gp1.

Supporting information

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

Table S1 Summary of the ddRADseq data.

Table S2 Selection of the optimal value of *K* from Structure.

Table S3 Summary of SNP and mtDNA data used for cline-fitting analyses.

Table S4 Frequency of foreign mtDNA haplotypes distributed across the hybrid zone.

Table S5 AIC model testing results for cline-fitting analyses.

Table S6 Temporal comparisons of ML cline-fitting results.

Fig. S1 Overview of SNP variation in the *Sceloporus tristichus* hybrid zone in Arizona using DAPC clustering of SNPs from replicated transect sampling conducted in (a) 2002 and (b) 2012.

Fig. S2 Mitochondrial DNA gene tree for *Sceloporus tristichus* and *S. cowlesi* estimated using ML. Numbers on nodes are bootstrap proportions. Samples from 2002 and 2012 are deposited at the AMNH and UWBM, respectively.

Fig. S3 Replicate ML cline-fitting analyses of the 2012 SNP data subsampled to match the 2002 sample size. (a) The ML clines for 50 replicate analyses superimposed. (b) Box plots of 50 replicate ML cline centre and width estimates, with arrows marking the full data estimates.

Fig. S4 Land cover stability in the centre of the hybrid zone. (a) Satellite image of the Sevenmile Draw sample locality taken in 1996. (b) Satellite image of Sevenmile draw taken in 2012. (c) Overlay of the 1996 satellite image over the 2012 image, with the 1996 image set to 50% transparent.