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Hybridization between multiple fence lizard lineages in an ecotone: locally discordant variation in mitochondrial DNA, chromosomes, and morphology

ADAM D. LEACHÉ* and CHARLES J. COLE†‡
Museum of Vertebrate Zoology and Department of Integrative Biology, 3101 Valley Life Sciences Building, University of California, Berkeley, CA 94720-3160, USA, †Department of Herpetology, American Museum of Natural History, New York, NY 10024-5192, USA

Abstract

We investigated a hybrid zone between two major lineages of fence lizards (*Sceloporus cowlesi* and *Sceloporus tristichus*) in the *Sceloporus undulatus* species complex in eastern Arizona. This zone occurs in an ecotone between Great Basin Grassland and Conifer Woodland habitats. We analysed spatial variation in mtDNA ($N = 401$; 969 bp), chromosomes ($N = 217$), and morphology ($N = 312$; 11 characters) to characterize the hybrid zone and assess species limits. A fine-scale population level phylogenetic analysis refined the boundaries between these species and indicated that four nonsister mtDNA clades (three belonging to *S. tristichus* and one to *S. cowlesi*) are sympatric at the centre of the zone. Estimates of cytonuclear disequilibria in the population closest to the centre of the hybrid zone suggest that the *S. tristichus* clades are randomly mating, but that the *S. cowlesi* haplotype has a significant nonrandom association with nuclear alleles. Maximum-likelihood cline-fitting analyses suggest that the karyotype, morphology, and dorsal colour pattern clines are all coincident, but the mtDNA cline is skewed significantly to the south. A temporal comparison of cline centres utilizing karyotype data collected in the early 1970s and in 2002 suggests that the cline may have shifted by approximately 1.5 km to the north over a 30-year period. The recent northward expansion of juniper trees into the Little Colorado River Basin resulting from intense cattle overgrazing provides a plausible mechanism for a shifting hybrid zone and the introgression of the mtDNA haplotypes, which appear to be selectively neutral. It is clear that complex interactions are operating simultaneously in this contact zone, including the formation of hybrids between populations within *S. tristichus* having diagnostic mtDNA, morphology, karyotypes, and dorsal colour patterns, and secondary contact between these and a distantly related yet morphologically cryptic mtDNA lineage (*S. cowlesi*).

Keywords: cline, cytonuclear disequilibrium, hybrid zone, phylogeny, *Sceloporus*, species limits

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Introduction

Geographic variation does not always manifest itself as gradual spatial change, but sometimes as sharp discontinuities over short geographical distances. These abrupt breaks in phenotypes and genotypes often occur at the interface between distinct habitats and are a result of either divergent selection across the environmental gradient or secondary contact following differentiation in isolation (Endler 1977; Barton & Hewitt 1985; Harrison 1993). Whether or not the homogenizing influence of gene flow accompanies divergence is a pivotal characteristic distinguishing these two modes of speciation. The absence of gene flow between sexual species at these contact zones is of particular importance, because reproductive isolation is a definitive indicator that the speciation process is complete. In this study, we investigate a contact zone between two

Correspondence: Adam D. Leaché, Fax: 510-643-8238; E-mail: leache@berkeley.edu
‡Present address: 6381 W. Sweetwater Drive, Tucson, AZ 85745, USA

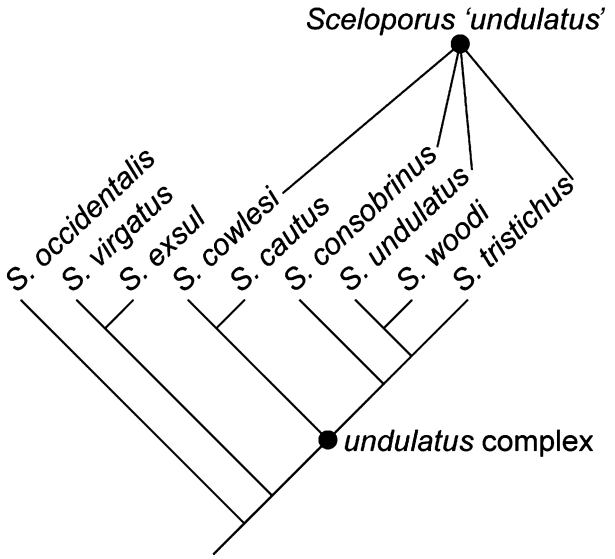

Fig. 1 Phylogenetic relationships within the *Sceloporus undulatus* species group based on mtDNA sequence data (Leaché & Reeder 2002). The taxa belonging to *S. 'undulatus'* (*sensu lato*) do not form an exclusive group and together with *S. cautus* and *S. woodi* form the *undulatus* complex. The placement of *Sceloporus exsul* is based on a phylogenetic analysis of morphological data (Wiens & Reeder 1997).

major mtDNA lineages of lizards in the *Sceloporus undulatus* complex by analysing spatial patterns of variation in mtDNA, chromosomes, and morphology.

The *S. undulatus* species group is a diverse assemblage of diurnal and insectivorous lizards distributed across North America. Although the species group is monophyletic, previous phylogenetic analyses based on morphology (Wiens & Reeder 1997), mtDNA (Wiens & Reeder 1997; Leaché & Reeder 2002), and allozymes (Miles *et al*. 2002) provided strong support for paraphyly of the most widespread and diverse member of the group, the Eastern Fence Lizard (*S. 'undulatus'*). Lizards comprising *S. 'undulatus'* exhibit a remarkable degree of geographical variation and are a model system for comparative studies in ecology and evolutionary biology (Angilletta *et al*. 2004; Niewiarowski *et al*. 2004; Cox *et al*. 2005; Rosenblum 2006; Angilletta *et al*. in press). This paraphyletic species complex contains *S. cautus*, *S. woodi*, and four divergent mtDNA haplotype clades that Leaché & Reeder (2002) found to be distinct evolutionary lineages and consequently recognized taxonomically as *S. consobrinus*, *S. cowlesi*, *S. tristichus*, and *S. undulatus* (Fig. 1). While it is important to characterize the genetic variation present in this species complex to provide a phylogenetic framework for comparative studies, we still lack an understanding of the evolutionary dynamics operating at any of the contact zones involving these mtDNA lineages. A contact zone between the *S. cowlesi* and *S. tristichus* mtDNA lineages in eastern Arizona provides a natural experiment to test the exclusivity of these lineages.

The contact zone between the *S. cowlesi* and *S. tristichus* mtDNA lineages in eastern Arizona coincides with an area of intergradation between two formerly recognized subspecies of *S. 'undulatus'*. The lizards inhabiting the Great Basin Grassland at the northern end of the contact zone differ markedly in size, colouration, and squamation from those that occur in Great Basin Conifer Woodland (Brown & Lowe 1980) to the south, and lizards with intermediate phenotypes are found in the canyon ecotones connecting these distinct habitats (Fig. 2). These morphological differences were characterized in the late 1800s and used to distinguish the Northern Plateau Lizard (*S. 'undulatus elongatus'* Stejneger 1890) from the Southern Plateau Lizard (*S. 'undulatus tristichus'* Cope, 1875), which we refer to as grassland and woodland ecomorphs, respectively. In the early 1970s, one of us (C.J.C.) identified hybrid lizards in the canyon ecotone using chromosome polymorphism data (Cole, unpublished). Since phylogenetic data presented by Leaché & Reeder (2002) suggested that the populations located to the north, south, and west of the contact zone are all nested within *S. tristichus*, it was unclear how hybridization between the grassland and woodland ecomorphs occurring along a north–south transect corresponds to an east–west contact zone between *S. cowlesi* and *S. tristichus* at the same location. This complex contact zone involves multiple interactions occurring simultaneously between mtDNA clades, chromosome groups, and ecomorphs, some or all of which may not actually correspond to unique evolutionary lineages, providing an excellent opportunity to study processes of speciation.

We present a detailed preliminary investigation of the *S. cowlesi* and *S. tristichus* contact zone in eastern Arizona with three main objectives. First, we conduct a fine-scale population level phylogenetic analysis to identify the boundaries and quantify the extent of overlap between the *S. cowlesi* and *S. tristichus* mtDNA lineages. Determining the spatial distribution of these lineages is critical for understanding how they correspond to the grassland and woodland ecomorphs hybridizing in the same area. Second, the chromosome polymorphism data add an important comparative aspect to our study, enabling us to assess the cytonuclear genetic structure of the hybrid zone and test for non–random associations between the nuclear and mitochondrial markers. Significant levels of cytonuclear disequilibria in a hybrid zone would indicate that some allelic combinations may experience epistatic effects on fitness or that nonrandom mating is occurring (Asmussen *et al*. 1987). Third, we use maximum-likelihood cline-fitting analyses to test the null hypothesis that there is no significant difference in the cline centres suggested by the mtDNA, chromosomes, morphology, and colour patterns of the lizards. Deviations from this expectation may suggest that neutral introgression, movement of the hybrid zone, or selection may be operating (Jaarola *et al*. 1997; Brumfield

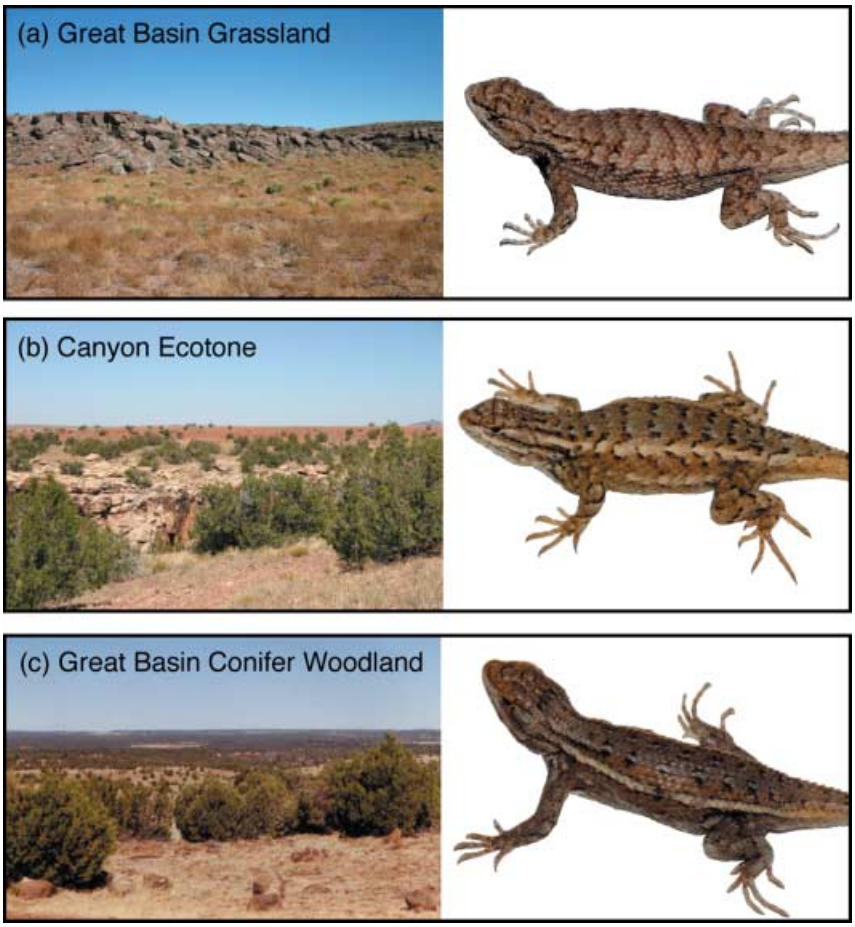

Fig. 2 Photographs of the habitat transition and the corresponding phenotypes of lizards through the contact zone. (a) The northern end of the contact zone is characterized by Great Basin Grassland. The grassland ecomorph is light brown to reddish in colour with dark, undulating cross-bars running across the back. (b) The lizards found in canyon ecotones are highly variable in colour and colour pattern and show a mixture of phenotypes expressed by the grassland and woodland ecomorphs, including vague dorsolateral light lines and dark lines running along the back that are an intermediate between paired dark and light spots and undulating crossbars. (c) The southern end of the contact zone is characterized by Great Basin Conifer Woodland. The woodland ecomorph is dark brown with paired light and dark spots running along the back and distinct dorsolateral light lines.

et al. 2001). We conclude by discussing which evolutionary processes are likely responsible for shaping this contact zone so we can more fully understand the nature of species boundaries in the *S. undulatus* complex.

Ecology of the contact zone

The hybrid zone is located in the Little Colorado River basin along Silver Creek, a tributary of the Little Colorado River located in Navajo County, Arizona, primarily between the cities of Snowflake and Holbrook (Fig. 3). Beginning at the southern edge of the contact zone south of Snowflake and moving north towards Holbrook the habitat transitions from Great Basin Conifer Woodland into Great Basin Grassland, a vegetation turnover evident primarily by a reduction in the abundance of juniper trees (*Juniperus* sp.) and increase in grasses. A lush, native grassland environment was pervasive throughout the Little Colorado River basin prior to the onset of intensive cattle ranching that began in the 1890s (Abruzzi 1995), which suggests a broader historical distribution of grassland lizard populations throughout the contact zone. Extensive cattle overgrazing resulted in a cascade of habitat alterations beginning with a drastic reduction in vegetative cover and an increase in surface water run-off, which in turn led to erosion and the formation of canyons and ravines (Abruzzi 1995). These factors are linked to the expansion of juniper into former grassland habitats across the southwestern USA (Archer 1994). Today, juniper trees are common along the rims of canyons and ravines as far north as Holbrook, although attempts over the years to remove junipers to promote a return to the original grassland environment has dramatically affected the appearance of the surrounding landscape (Abruzzi 1995).

Materials and methods

Mitochondrial DNA and phylogenetic analysis

We collected mtDNA sequence data for 360 *Sceloporus cowlesi* and *Sceloporus tristichus* from throughout the southwestern USA, with an emphasis on the vicinity of the contact zone in eastern Arizona (Fig. 3; Appendix I). A total of 161 samples represent specimens collected from 15 localities within the contact zone, each containing sympatric haplotypes belonging to either *S. cowlesi* or *S. tristichus*, with sample sizes

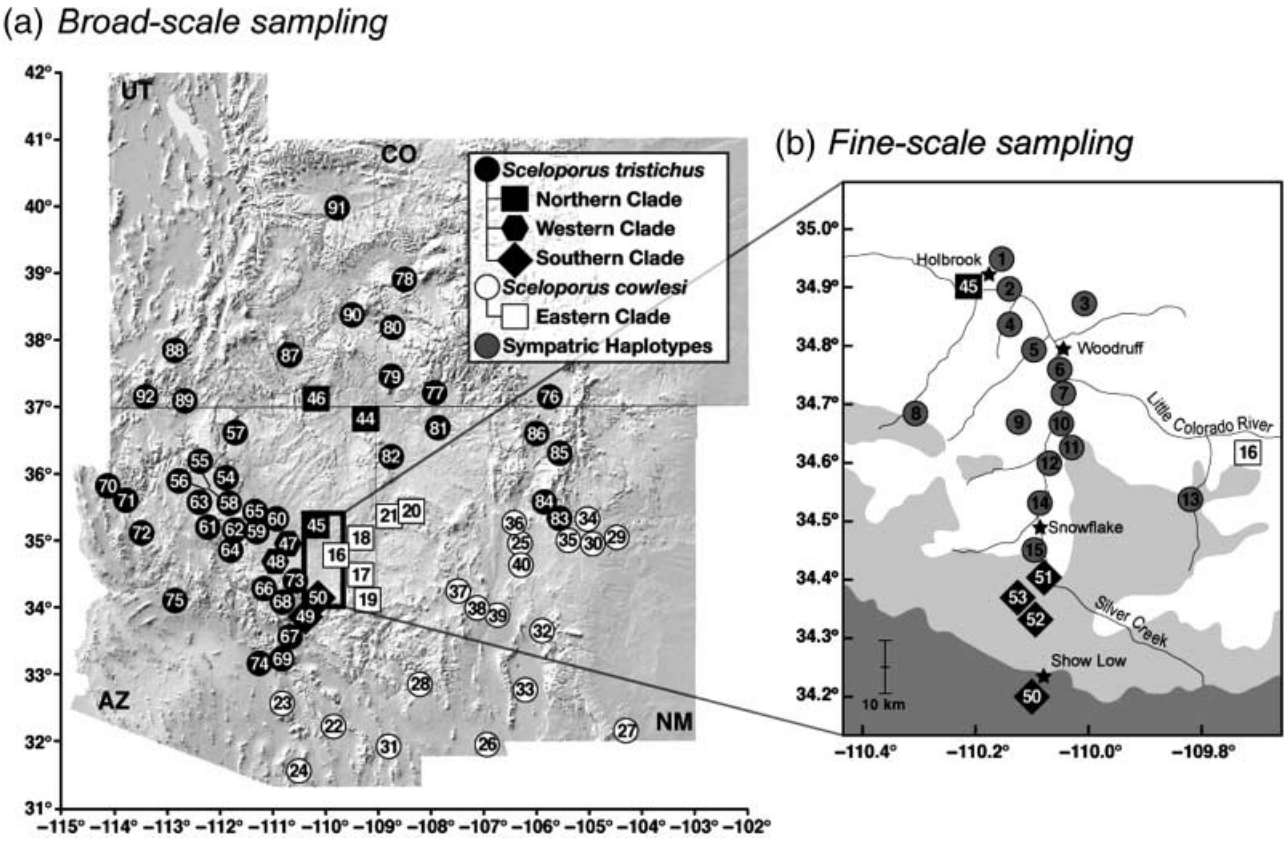

Fig. 3 Maps of the study areas. (a) Broad-scale sampling of specimens throughout the southwestern USA. Numbered localities are listed in Appendix I and represent sample sites of *Sceloporus cowlesi* and *Sceloporus tristichus* included in the mitochondrial DNA gene tree. Subclades of *S. cowlesi* and *S. tristichus* are named according to their orientation with respect to the contact zone (located in the black rectangle). (b) Fine-scale sampling of specimens through the contact zone. Localities in the contact zone containing sympatric haplotypes belonging to *S. cowlesi* and *S. tristichus* are designated with grey circles. The distributions of Petran Montane Conifer Forest, Great Basin Conifer Woodland, and Great Basin Grassland habitats are shown in dark grey, light grey, and white, respectively.

ranging from 2 to 43 individuals per locality (average = 10.7; Appendix I). The additional 199 samples describe the geographical variation of these lineages throughout the southwestern USA and north-central Mexico. We also included 41 representatives of the remaining *S. undulatus* group species (i.e. *S. cautus*, *S. consobrinus*, *S. occidentalis*, *S. undulatus*, *S. virgatus*, and *S. woodi*), resulting in a final data matrix containing 401 specimens. Seventy-two of our samples were used in a previous phylogenetic analysis of the *S. undulatus* group (Leaché & Reeder 2002).

Total genomic DNA was isolated from fresh tissue samples (liver or muscle) using QIAGEN DNeasy extraction kits (QIAGEN Inc.). We sequenced the entire mitochondrial ND1 protein coding gene (969 bp), because these data are combinable with data collected previously and this gene exhibits more variation compared to 12S and 16S ribosomal RNA genes (Leaché & Reeder 2002). We developed lineage-specific polymerase chain reaction (PCR) primers (SnowF 5′GCAGAGCCAGGTTTATGCACAAGC; SnowR 5′GTGCAGGTTCAAGTCCTGTTCTTC) to amplify the entire ND1 gene. Approximately 10–25 ng of total DNA was used as template for PCR in a final volume of 25 μL containing 1× buffer, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.5 μM of each primer, and 1.25 U of *Taq* polymerase. Sufficient PCR product for direct sequencing was generated after 36 cycles (94 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min). PCR products were purified using QIAGEN PCR purification kits (QIAGEN Inc.). Purified templates were sequenced using dye-labelled dideoxy terminator cycle sequencing with BigDye version 3.1 (Applied Biosystems, Inc.) and run on an ABI PRISM 377 automated DNA sequencer.

Contiguous sequences of DNA were aligned and edited using SEQUENCHER version 4.2. The protein-coding ND1 sequences lacked length variation and were imported directly into NEXUS file format for phylogenetic analyses. Unique mitochondrial DNA sequences are deposited in GenBank (accession nos EF031564–EF031924).

To test for deviations from neutrality in the mtDNA data, we employed the McDonald–Kreitman test (McDonald & Kreitman 1991) and calculated Tajima's *D* (Tajima 1989) as implemented in the program DNASP version 4.10.4 (Rozas

et al. 2003). For the phylogenetic analyses, we used a partitioned Bayesian approach including all unique haplotypes using MRBAYES version 3.0b5 (Huelsenbeck & Ronquist 2001), with three partitions corresponding to first, second, and third codon positions (Brandley *et al*. 2005). To select the best-fit nucleotide substitution model for each data partition, we used the Akaike information criterion implemented in MRMODELTEST version 2.2 (Nylander 2004).

We produced a posterior probability distribution by allowing four incrementally heated Markov chains (default heating values) to proceed for 4×10^7 generations (sampling every 1000 generations). To determine burn-in, we plotted the cumulative posterior probabilities of nodes from the Markov chain Monte Carlo (MCMC) runs using the online convergence program 'Are We There Yet' (AWTY; Wilgenbusch *et al.* 2004). To further ensure that our analyses were not restricted to local optima, we plotted the posterior probabilities of all splits from two separate MCMC runs. Burn-in samples were discarded from the two separate analyses, and the remaining samples were combined to produce a 50% majority rule consensus tree with bi-partition frequencies equal to posterior probability values (Huelsenbeck & Ronquist 2001). We rooted our tree using *Sceloporus occidentalis* based on the relationships presented in Leaché & Reeder (2002). Since we are interested primarily in refining the mtDNA clade boundaries between *S. cowlesi* and *S. tristichus* and determining their extent of overlap, we condensed the phylogeny by omitting details pertaining to the relationships within species that are not participating in the contact zone.

Chromosome polymorphism and cytonuclear disequilibria

Cole (1972) described an apparent pericentric inversion polymorphism on chromosome seven in the *S. undulatus* group. Four distinct inversions were discovered across the range of *S. 'undulatus'* and every individual examined within a given population was fixed for just one inversion variant, providing evidence that the position of the centromere is heritable and can be used as a diagnostic tool for identifying or characterizing populations (Cole 1972). The polymorphism of this character is based on the location of the centromere along the chromosome, and three character states are found in the hybrid zone; telocentric (T): a terminal or essentially terminal centromere; subtelocentric (ST): a nonterminal centromere that is closer to one end than to the middle of the chromosome; submetacentric (SM): a nonmedial centromere that is closer to the middle than to either end of the chromosome (Cole 1972). Given that theoretically mutations can shift the centromere to any position along a chromosome and that differences between centromere positions may be so subtle as not to be resolved by our methods, our categories may artificially treat essentially continuous patterns of variation as discrete and thus may underestimate the variation in the hybrid zone. As in all vertebrates, however, in the absence of mutation centromere positions are inherited unchanged.

We prepared slides and scored mitotic chromosomes for 101 individuals collected from the contact zone in 2002, and one of us (C.J.C.) prepared 116 lizards between 1971 and 1976. The locality data for the 217 specimens scored for the karyotype polymorphism are provided in Appendix II. Field captured lizards were transported to the Southwestern Research Station (American Museum of Natural History) where they were weighed and injected with 0.10–0.20 mL of a yeast/sugar solution to elicit an immune response and stimulate cell division (Cole & Leavens 1971). At approximately 18 h after injection and incubation at 30 °C the lizards were injected with 0.01 cc/gram of body weight of 0.05% colchicine to arrest mitotic cell division. Lizards were sacrificed with Nembutal 5 h after the colchicine injection, and cells were harvested from testes and bone marrow from femurs and tibias. Slides were prepared following the protocol of Cole (1972). Chromosomes were visualized at 1000× oil immersion, and we scored at least four metaphase-cells with condensed chromosomes per lizard to determine the character state of chromosome seven.

We tested for cytonuclear disequilibrium in this multiallelic system using the program CNDM (Asmussen & Basten 1996; Basten & Asmussen 1997). Cytonuclear disequilibrium (D_M^A) is the nonrandom association between genotypes or alleles at nuclear and cytoplasmic (mtDNA or cpDNA) loci (Arnold 1993) within a population. We were only able to utilize the karyotype and mtDNA data collected during 2002 in our analysis of cytonuclear disequilibrium because we lacked mtDNA data for specimens collected during the 1970s. In this system, we treat the three chromosome character states (T, ST, and SM) as nuclear alleles and the mtDNA clades as cytoplasmic alleles. We characterized the disequilibria at the centre of the contact zone (locality #10) where our sampling of individuals for mtDNA and chromosomes was highest ($N = 26$). Significance of allelic disequilibria was assessed using Fisher's exact test (Basten & Asmussen 1997).

Morphometrics, colour patterns, and multivariate statistical analysis

Differences in the dorsal colour patterns of the grassland and woodland populations of lizards in the contact zone are apparent (Fig. 2), but it is unclear if any differences in morphology separate these populations. In order to test for a correspondence between variation in dorsal colour patterns and morphology we assembled a morphometric data set containing nine characters from 312 specimens. Voucher specimen information for all specimens included in this study are provided in Appendix III. The following mensural characters were recorded from each specimen

using digital calipers to the nearest 0.1 mm: standard length from the tip of the snout to the anterior margin of the vent (SVL); head length from the anterior tip of the rostral scale to the posterior margin of the interparietal scale (HL); maximum width of the head at the level of the last supralabials (HW); length of the fourth toe from the tip of the claw to the base by the third toe (TL). The following meristic characters were counted using a dissecting microscope: number of dorsal scales along the middorsal region from the interparietal to the posterior margins of the thighs (DS); number of subdigital lamellae summed from the left and right fourth toes of each hind foot (LAM); number of femoral pores summed from the left and right hind legs (FEMS). We also scored two ventral colour characters from digitized images of specimens captured using a flatbed scanner (CanoScan LiDE30, Canon Inc.). Ventral colouration characters included the area covered by the coloured throat patches (THAR) and the area covered by an abdominal colour patch (ABAR). We size corrected ABAR and THAR using SVL and HW, respectively. We quantified the areas covered by ventral pigmentation by standardizing the resolution of the digital images at 100 pixels/inch and quantifying the number of coloured pixels encompassed by the patches (determined in ADOBE PHOTOSHOP 7.0; Adobe Systems Inc.).

We scored two dorsal colour pattern characters to quantify the differences in the grassland and woodland populations. The two characters included the intensity of dorsolateral light lines and the presence of paired light and dark spots or undulating crossbars on the back (Fig. 2). We scored each of these characters on a scale of 1–5 as follows: 1 and 5 represent the 'pure' southern/woodland and northern/grassland conditions, respectively; 3 represents a 'hybrid' state halfway between the 'pure' southern and northern conditions; and 2 and 4 represent intermediate conditions between the 'pure' southern/woodland to 'hybrid' and 'pure' northern/grassland to 'hybrid' states, respectively.

We conducted statistical analyses using SYSTAT version 8.0. We performed principal component analyses (PCA) and canonical discriminant function analyses (DFA) after log-transforming the mensural data. We excluded juveniles to eliminate any bias in size as a result of allometric growth. Since these lizards exhibit sexual dimorphism in size, colour, colour pattern, and squamation, we analysed male and female data separately. We performed PCA analyses to test whether morphological groupings are discernable without the designation of any a priori hypothesis of group membership. We performed the DFA analysis to test whether it is possible to differentiate among a priori-defined ecomorph groups (woodland vs. grassland populations) using the morphometric data. The dorsal colour pattern characters were excluded from the PCA and DFA analyses because we treat dorsal colour pattern and morphology separately in the cline fitting analysis. We used the following latitudinal cut-off values to define the ecotone boundaries where habitat differences separating the ecomorphs become ambiguous: woodland localities < 34.5° grassland localities > 34.9° hybrid localities between 34.5° and 34.9°.

Cline fitting analysis

Our one-dimensional transect runs from north to south following Silver Creek, starting in Holbrook to the north and terminating near Show Low. Some localities within several kilometres of each other were grouped together to increase sample sizes, resulting in nine localities spanning 70 km (Table 1). We analysed four character clines including mtDNA, chromosomes, morphology, and dorsal colour pattern. Character clines were scaled from 0 and 1 to represent 'pure' northern and southern populations, respectively. For the cline-fitting analysis of the mitochondrial data we only considered the northern and southern haplotypes since these are fixed at the tails of the transect. We plotted the frequencies of the eastern and western

Table 1 Summary of genetic and morphological data used to analyze a north to south transect through the hybrid zone. Frequency values are scaled from 0 to 1 to represent 'pure' northern and southern populations, respectively. Distance is from the northernmost locality

		mtDNA		Chromosomes (1970s + 2002)		Chromosomes (2002)		Morphology		Colour pattern	
Locality	Distance (km)	*N*	Freq.	*N*	Freq.	*N*	Freq.	*N*	Freq.	*N*	Freq.
1, 2, 45, 94	0	13	0.08	26	0.00	18	0.00	12	0.22	9	0.00
4	10	8	0.00	32	0.00	14	0.00	21	0.52	5	0.19
3, 5	16	11	0.00	13	0.00	13	0.00	7	0.07	7	0.33
6	24	5	0.00	4	0.00	2	0.00	6	0.3	5	0.38
7	27	4	0.00	2	0.00	2	0.00	4	0.00	4	0.23
9, 10, 95	32	25	0.28	123	0.17	39	0.03	52	0.45	15	0.38
11, 12, 96	39	12	0.17	36	0.92	13	1.00	15	0.67	6	0.71
14	48	5	1.00	12	1.00	12	1.00	7	0.86	7	0.87
52	70	12	1.00	18	1.00	18	1.00	7	1.00	7	1.00

haplotypes separately since we could not include them in the formal cline-fitting analysis. We conducted two separate analyses of the karyotype data to test whether the centre of the contact zone has moved over our 30-year sampling period. The first analysis included only the karyotype data collected in 2002, while the 1970s and 2002 data were pooled for the second analysis. Although the number of karyotypes collected in the 1970s is greater than our 2002 sample, we could not conduct an exclusive analysis of the 1970s data because the number of populations corresponding to our 2002 sampling transect is too low (Appendix II). Therefore, we may consider any differences we detect in cline centres as minimum estimates of movement, since the 2002 data can influence the cline position suggested by the 1970s data, yet results of this analysis may be useful to guide future sampling. For the chromosomal data we calculated the frequency of the subtelocentric chromosome separately and excluded it from the cline fitting analysis, since the submetacentric and telocentric chromosomes are fixed at the tails of the transect. The population means for dorsal colour patterns and the PC1 score from the morphometric data set were scaled to values between 0 and 1 using the equation (population mean – min. mean)/(max. mean – min. mean).

We fitted tanh curves through the mtDNA, chromosomes, morphology, and dorsal colour pattern cline data using the 'Fit 1D Cline' option in the program ANALYSE version 1.3 (Barton & Baird 1999). Model parameters were estimated by running 2000 iterations from 20 different starting points. We used the 'cross section' option to determine the two log-likelihood support limits for parameter estimates. We tested for significant discordance in cline coincidence using a maximum-likelihood cline-fitting procedure (Brumfield *et al*. 2001; Phillips *et al*. 2004). For each data set, we explored the likelihood surface of cline centre at intervals of 100 m while allowing the cline width to vary freely. A likelihood ratio test was used to statistically compare the maximum log-likelihood support of a constrained model (L_c) that assumed cline coincidence among the data sets to the log-likelihood support of an unconstrained model (L_u) that selected the maximum-likelihood score for cline centre from each data set. The test statistic Δ was calculated as two times the absolute difference in log-likelihood between the L_c and L_u models, and significance was determined by comparison to the χ^2 distribution ($\alpha = 0.05$) with the degrees of freedom equal to the difference in the number of parameters estimated in the models.

Results

Mitochondrial DNA and phylogenetic analysis

The 360 complete ND1 mtDNA sequences (969 bp) for *Sceloporus cowlesi* and *Sceloporus tristichus* contained 150 unique haplotypes and exhibited no length variation. The majority of the redundant haplotypes were distributed throughout the contact zone in eastern Arizona where our sampling was most dense. The maximum uncorrected 'p' sequence divergence of the 55 localities represented by multiple samples was 10.63% (range: 0.00–10.63%; Appendix I). The average maximum sequence divergences at localities inside vs. outside the contact zone are 8.84% and 0.626%, respectively.

The results of the McDonald–Kreitman test and Tajima's *D* were not significant, indicating that the mtDNA data are evolving under neutral expectations. The general time-reversible model with some sites assumed to be invariable and with variable sites following a discrete gamma distribution (i.e. GTR + I + Γ Yang 1994) was selected as the best-fit model of nucleotide substitution for the first and third codon positions of the *ND1* data, and the less parameter-rich GTR + I model was selected for the more slowly evolving second codon positions. Convergence of the Bayesian analyses appeared complete by 2×10^6 generations based on an inspection of a burn-in plot of log-likelihood values, but this was delayed by nearly an order of magnitude (1×10^7 generations) when considering the burn-in of cumulative posterior probability values of nodes. Therefore, we discarded the first 2000 samples from each analysis, resulting in two posterior distributions containing 2000 samples each. A plot of the posterior probabilities of all splits from the two separate MCMC runs demonstrated a linear relationship, suggesting that our analyses were not restricted to local optima.

The Bayesian phylogeny inferred for the *Sceloporus undulatus* species group placed the newly collected samples from throughout the south-western U.S. into the *S. cowlesi* and *S. tristichus* lineages (Figs 4, 5). The phylogeny provides strong support ($P = 1.0$) for the placement of *S. cowlesi* and *S. cautus* sister to a clade containing *S. consobrinus*, *S. tristichus*, *S. undulatus*, and *S. woodi*, although significant support is not provided for the interrelationships among these species (Fig. 4). A lack of significant support for these particular nodes was also found by Leaché & Reeder (2002) based on an unpartitioned Bayesian analysis of ND1 data, suggesting that support for these nodes requires additional data and not just more appropriate partitioning strategies or increased taxon sampling. We emphasize that the *S. cowlesi* and *S. tristichus* mtDNA lineages are not sister taxa, since *S. tristichus* is nested with *S. consobrinus*, *S. undulatus*, and *S. woodi*.

Sceloporus cautus is placed within *S. cowlesi*, although strong support is not provided to contradict *S. cowlesi* monophyly. Only one clade within *S. cowlesi* contains haplotypes found in sympatry with *S. tristichus* (Fig. 4). This clade, termed the eastern clade based on its orientation to the contact zone, is distributed from the contact zone to west-central New Mexico (Figs 3 and 4). The eastern clade

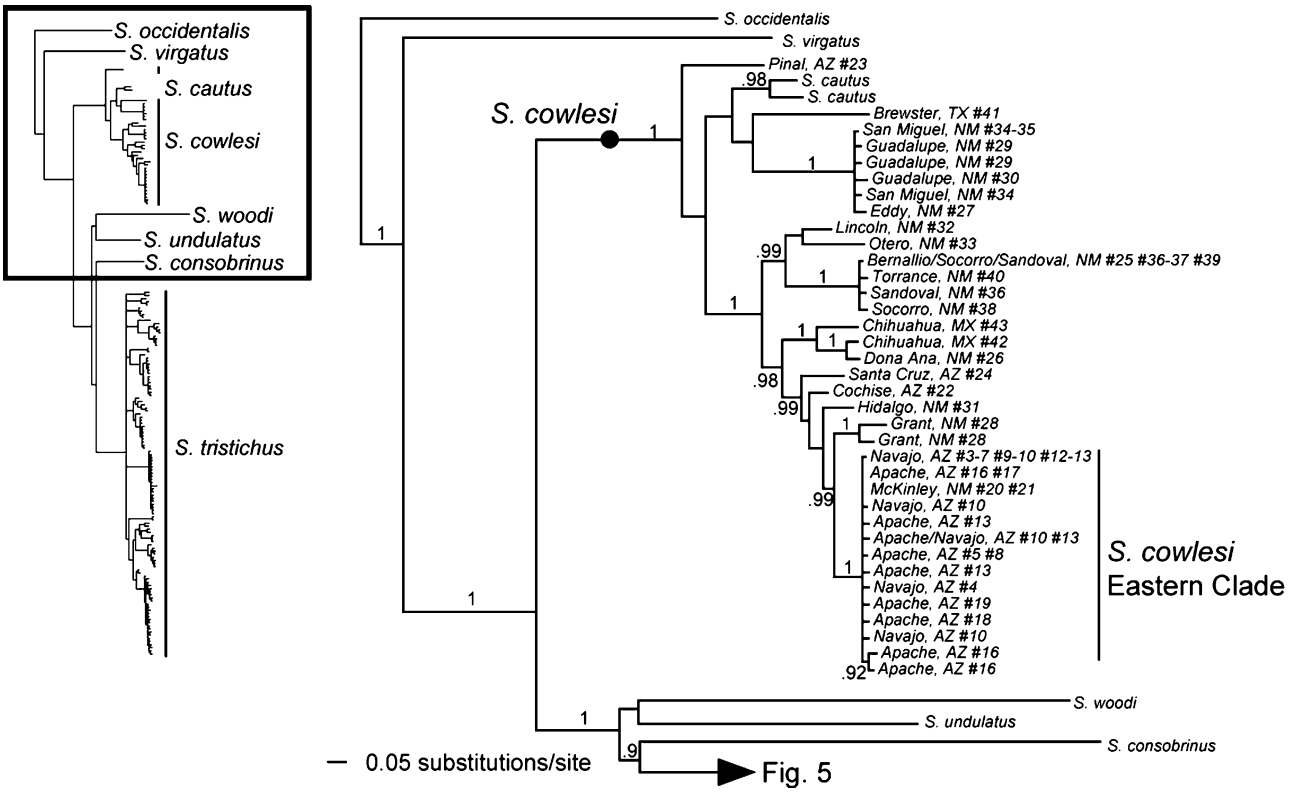

Fig. 4 Bayesian 50% majority-rule consensus tree of the ND1 sequence data (969 bp) for *Sceloporus cowlesi*. The *S. cowlesi* eastern clade contains haplotypes that are sympatric with three *S. tristichus* clades, and is named according to its geographical orientation to the contact zone. Numbers on nodes correspond to posterior probability values, and only those nodes supported at 0.90 or greater are labelled. Locality numbers are provided next to the terminal taxa and detailed in Appendix I.

is sister to a series of haplotype clades distributed throughout southwestern New Mexico and Arizona (Fig. 4).

Sceloporus tristichus is composed of many strongly supported mtDNA clades restricted to relatively small geographical areas (Fig. 5). The relationships among these clades are ambiguous, resulting in a large polytomy at the base of the *S. tristichus* phylogeny (Fig. 5). The *S. tristichus* haplotypes discovered in the contact zone fall into three nonsister clades (Fig. 5). These clades are named according to their geographical orientation relative to the contact zone (i.e, northern, western, and southern clades; Fig. 3). Strong support unites the southern clade with populations occurring in the southwestern portion of the *S. tristichus* range, while the western clade is nested among populations extending from the contact zone through northern Arizona and southern Utah (Figs 3 and 5). The northern clade stems directly from the polytomy at the base of the *S. tristichus* phylogeny, and contains haplotypes that extend as far north as San Juan Co., Utah (Figs 3 and 5).

Fine-scale mapping of mtDNA haplotypes in the contact zone reveals localities with mixed haplotypes extending from just south of Snowflake (locality #15) to Holbrook (locality #1), with one locality along Silver Creek (locality #10) containing sympatric haplotypes belonging to all four haplotype clades (i.e. *S. tristichus* northern, western, southern clades, and *S. cowlesi* eastern clade; Fig. 6a). Surprisingly, haplotypes belonging to the western clade were not found in the western-most localities of the contact zone, but instead only appeared at four localities along Silver Creek and two localities west of the contact zone (localities #47 and #48; Fig. 3). The cause of this hiatus in the distribution of western clade haplotypes is unclear, but could be a sampling artifact. Overlap between *S. cowlesi* and the southern *S. tristichus* clade was only detected as far as ~ 20 mi east of the contact zone (Fig. 6a).

Chromosome polymorphism and cytonuclear disequilibrium

The local polymorphism in chromosome number seven is extensive in the contact zone given that this chromosome is monomorphic in all other populations of the *S. undulatus* complex examined from across the United States (Cole 1972 and more recent unpublished data). We found three distinct centromere positions for chromosome seven in the contact zone corresponding to telocentric (T), subtelocentric

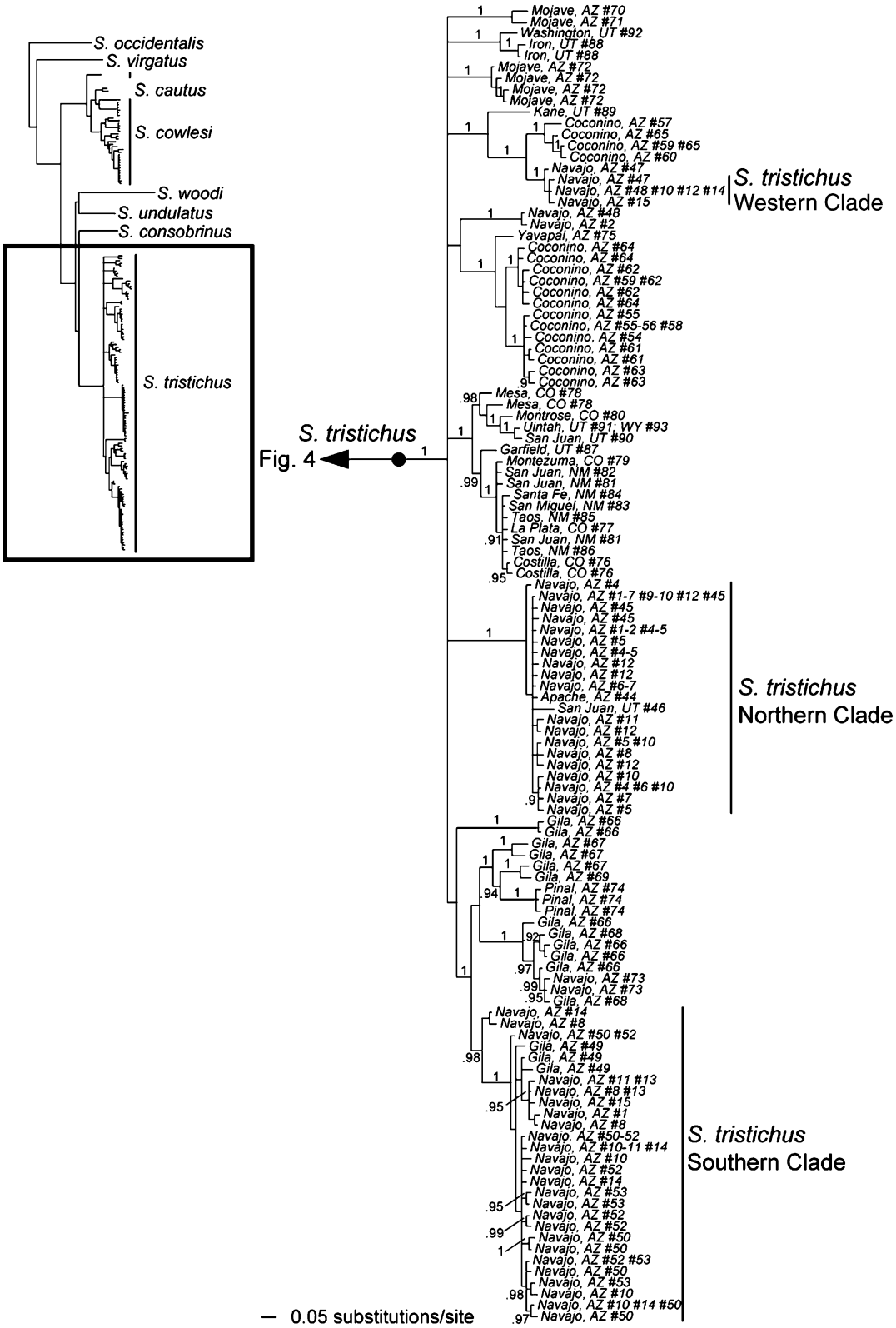

Fig. 5 Bayesian 50% majority-rule consensus tree of the ND1 sequence data (969 bp) for *Sceloporus tristichus*. The northern, southern, and western subclades of *S. tristichus* are sympatric with the eastern *Sceloporus cowlesi* clade, and are named according to their geographical orientations to the contact zone. Numbers on nodes correspond to posterior probability values, and only those nodes supported at 0.90 or greater are labelled. Locality numbers are provided next to the terminal taxa and detailed in Appendix I.

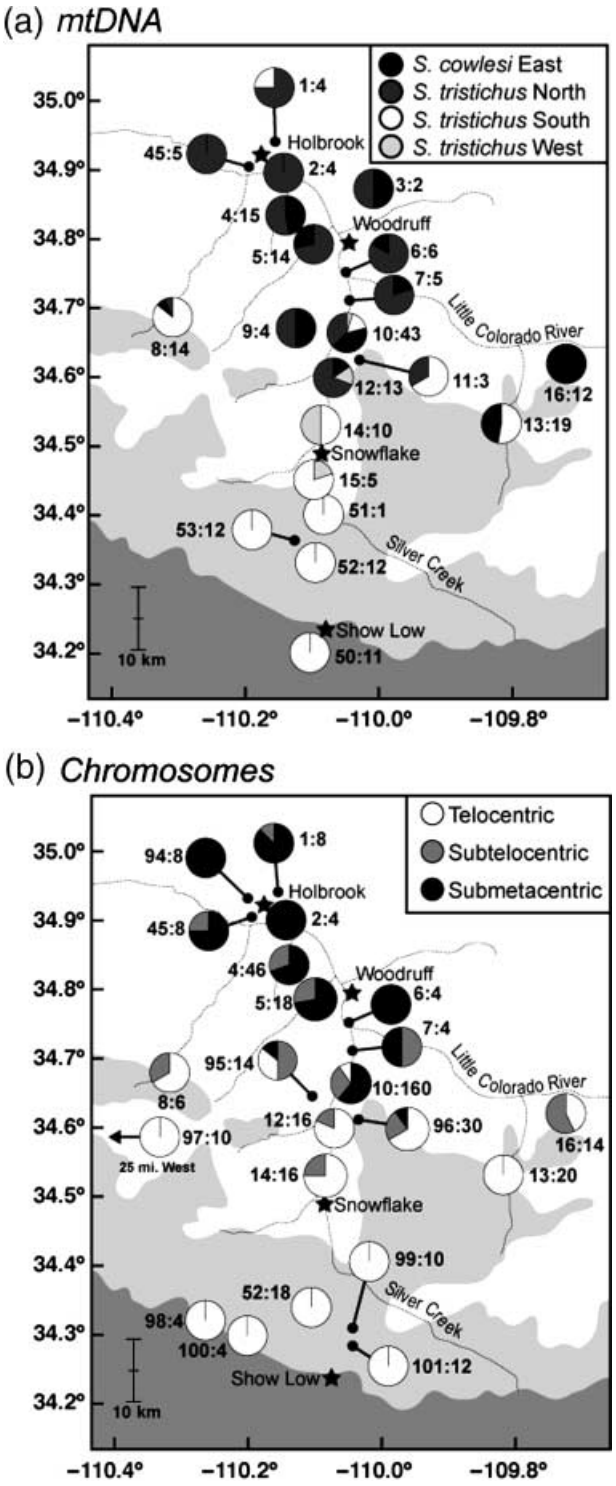

Fig. 6 Frequency of (a) mitochondrial DNA haplotypes and (b) chromosome number seven variants (treated as alleles) at localities sampled in the contact zone. Numbers adjacent to pie-charts correspond to the locality number (see Appendix I and Appendix II), followed by the sample size. The distributions of Petran Montane Conifer Forest, Great Basin Conifer Woodland, and Great Basin Grassland habitats are shown in dark grey, light grey, and white, respectively.

(ST), and submetacentric (SM) character states (Table 2). All possible combinations between these three states are present in the contact zone (i.e. T + T, ST + ST, SM + SM, T + ST, T + SM, ST + SM), and locality #10 along Silver Creek contained all six chromosomal combinations (Appendix II; Fig. 6b). The SM chromosome is distributed throughout the north (Holbrook area), and the T chromosome is more broadly distributed throughout the south, west, and east (Show Low, Snowflake, and localities #97 and #13; Fig. 6b). These two chromosomes are only found in sympatry along Silver Creek at localities #10, #95 and #96 (Appendix II; Fig. 6b). The ST chromosome is distributed throughout the contact zone, but no pure ST + ST populations were found in the area (Fig. 6b). Individuals with heteromorphic pairs of chromosomes are often found in populations that otherwise only contain their presumptive parental chromosomes, such as localities #4 and #5 (SM + SM, ST + ST, ST + SM) and localities #14 and #16 (T + T, ST + ST, T + ST).

Reed *et al*. (1990) hypothesized that chromosome number seven might be the X and Y sex chromosomes in *Sceloporus 'undulatus'* from Utah, but we have seen no evidence of this in our data. In the Silver Creek contact zone, individuals of both sexes have the six possible permutations of chromosome seven combinations with respect to centromere positions. We examined testicular cells of chromosomal heterozygotes at meiosis I and meiosis II and found no evidence of meiotic failure. Cells at meiosis I (diakinesis and metaphase I) showed only apparently normal bivalents ($N = 27$ cells examined in seven heterozygous lizards). Heterozygotes sorted out appropriately at prophase II and metaphase II for the following seven lizards: AMNH 108124 = 8 SM cells +10 ST cells; AMNH 108125 = 2 SM +3 ST; AMNH 111121 = 3SM +7T; AMNH 111124 = 6 SM +11 ST; AMNH 112479 = 1 SM +1T; AMNH 112480 = 1 SM +1ST; and AMNH 112492 = 2 SM +3T.

We detected a significant nonrandom association between nuclear genotypes and mitochondrial haplotypes at the centre of the contact zone in population #10 (Table 2). The *S. cowlesi* haplotypes have a significant nonrandom association with the SM and ST chromosomes, different character states in centromere positions being treated as alleles (Table 2, $P < 0.05$; Fisher's exact test). No significant estimates of cytonuclear disequilibria were provided for *S. tristichus*, suggesting that the nuclear alleles belonging to subclades of this lineage are in equilibrium at the centre of the contact zone.

Morphometrics, colour patterns, and multivariate statistical analysis

A list of descriptive statistics for the characters used in the multivariate analyses is provided in Appendix IV, and multivariate statistical results are presented for male lizards only. The first four principle components (PC I–IV)

		Mitochondrial cytotype		
		Sceloporus tristichus		*Sceloporus cowlesi*
		North	South	East
Nuclear allele	Submetacentric	−0.0311 (0.3470)	−0.0429 (0.1611)	0.0740 **(0.0191)**
	Telocentric	−0.0074 (1.0000)	0.0141 (0.2692)	−0.0067 (1.0000)
	Subtelocentric	0.0385 (0.2079)	0.0288 (0.2996)	−0.0673 **(0.0210)**

Table 2 Cytonuclear allelic disequilibrium (D_M^A) for the chromosome seven variants and mitochondrial cytotypes at the center of the hybrid zone (population #10). Probabilities from Fisher's exact test are shown in parentheses, and P values < 0.05 are shown in bold

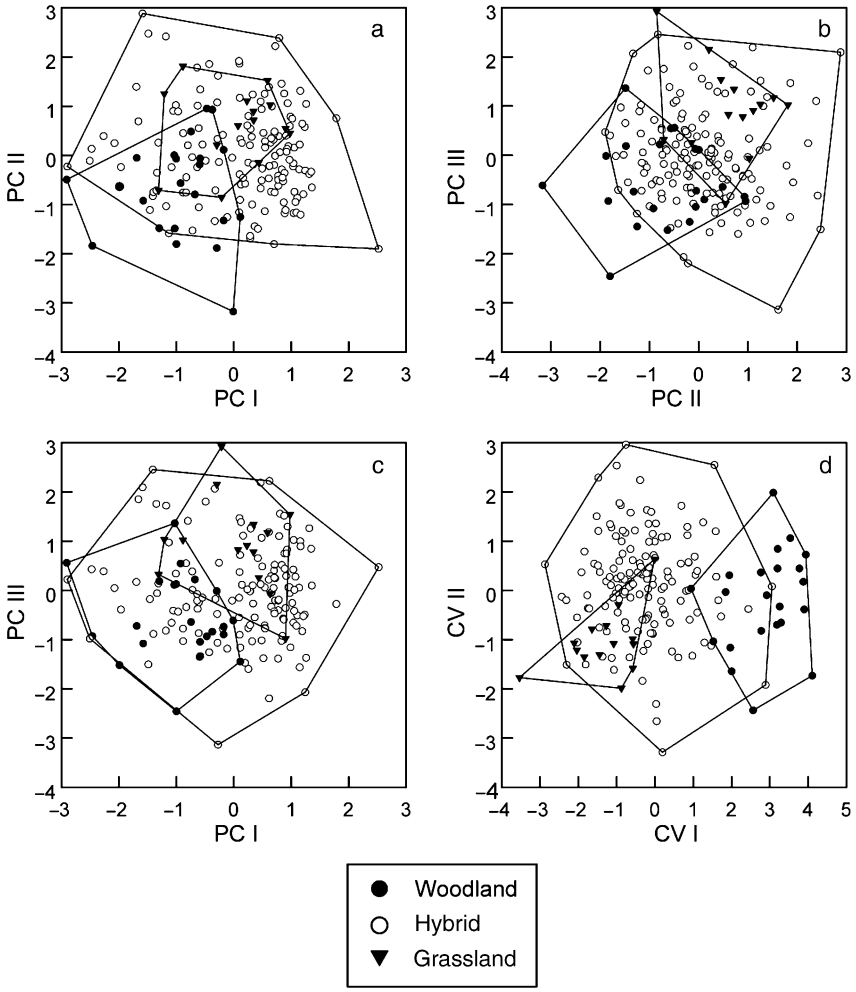

Fig. 7 Multivariate plots of the morphometric data showing the first three principal components (a—c) and two canonical variate scores (d). Symbols are as follows: woodland localities (filled circles); grassland localities (filled triangle); hybrid localities (unfilled circles). Only results for males are shown.

account for over 92% of the variation in our PCA analysis. The loadings for PC I are large and positive, indicating that PC I is a size variable. All of the meristic variables on PC II are negative and load heavily, and the loadings for the pigmentation characters are negative on PCIII. The grassland and woodland lizards are not entirely separated from one another in the PCA plots, and the lizards from the hybrid zone occupy a broad region of morpho-space, which encompasses that of the ecomorphs (Fig. 7a–c). Our DFA clearly discriminates between the grassland and woodland lizard ecomorphs (Fig. 7d), with CV I containing over 92% of the total variation.

Cline fitting analysis

A likelihood ratio test rejects the hypothesis that the mtDNA, chromosomes, morphology, and dorsal colour pattern clines are not significantly different ($\chi^2 = 10.33$, d.f. = 3, $P < 0.05$; Table 3). When the mtDNA data are excluded, the hypothesis that the remaining character clines are coincident cannot

Table 3 Cline parameters and likelihood-ratio test of the null hypothesis that the cline centers are not significantly different from each other. Cline center and width are shown in units of kilometres, and support limits (two log-likelihood units) are shown in parentheses. ln L_u is the unconstrained log-likelihood support. ln L_c is the maximum constrained log-likelihood from an analysis in which the cline center was held constant at 100-m intervals spanning the contact zone while other parameters were free to vary. The null hypothesis that all four cline centers are coincident is rejected ($\chi^2 = 10.33$, d.f. = 3; critical χ^2 value at $P = 0.05$ is 5.99). When the mtDNA data are excluded from the analysis (shown in brackets) the null hypothesis cannot be rejected ($\chi^2 = 1.55$, d.f. = 2; critical χ^2 value at $P = 0.05$ is 5.99). Separate analyses using the combined chromosome data set and the 2002 only data produced similar results (the combined data results are shown)

	mtDNA	Character Chromosomes (1970s + 2002)	Chromosomes (2002 only)	Morphology	Colour pattern
Cline Center	41.8 (36.8–43.8)	34.8 (33.8–36.0)	33.32 (32.02–36.8)	30.1 (21.1–41.0)	32.2 (25.6–40.0)
Cline Width	30.9 (26.9–43.8)	6.9 (5.6–8.8)	1.5 (0.0–5.9)	89.5 (115.2–139.9)	41.4 (46.4–55.8)
ln L_u	−7.61	−0.04	−0.00	−9.03	−1.99
Σ ln L_u	−18.67 [−11.06]				
Σ ln L_c	−23.835 [−11.83]				
$\Delta = \Sigma \ln L_c - \Sigma \ln L_u$	5.165 [0.77]				
$\chi^2 = 2\Delta$	10.33 [1.55]				

be rejected ($\chi^2 = 1.55$, d.f. = 2, $P > 0.05$; Table 3). A visual inspection of cline shapes shows that the mtDNA cline is shifted significantly to the south (Fig. 8). The maximum likelihood estimate for the position of the cline centre excluding the mtDNA data is 34.7 km south of Holbrook, just south of population #10, while the cline centre for the mtDNA data is 41.8 km south of Holbrook. Separate temporal analyses of the karyotype data (combined 1970s and 2002 vs. 2002 only) suggest a slight shift in the centre of the cline and change in cline width (Table 3). The shift in the cline centre is approximately 1.5 km to the north in the 2002 only data, and the cline width is reduced from 6.9 km to 1.5 km, but the two log-likelihood support limits surrounding these estimates are overlapping (Table 3).

The distribution of haplotypes belonging to the western *S. tristichus* clade is limited to just three populations at the centre of the contact zone (Fig. 8a). The highest frequency of the western haplotypes is 50% in population #14, which is located to the south of the contact zone. *Sceloporus cowlesi* haplotypes are found in the centre and north of the contact zone, but are not found to the south where the southern and western *S. tristichus* subclades occur (Fig. 8a). The subtelocentric chromosome variant is distributed throughout the northern end of the transect and also occurs in two populations located to the south of the inferred cline centre, but does not appear to reach the southernmost localities sampled (Fig. 8b).

Discussion

Hybridization within Sceloporus tristichus *and the influx of* Sceloporus cowlesi *haplotypes*

Hybrid zones are areas where two populations of individuals that are distinguishable on the basis of one or more heritable characters overlap spatially and temporally and interbreed to form viable and at least partially fertile offspring (Arnold 1997). The *Sceloporus cowlesi* and *Sceloporus tristichus* contact zone contains four sympatric mtDNA clades, some of which interbreed to form hybrids. Hybrid zones usually involve only two distinct parental forms (e.g. Dessauer *et al*. 2000), and other studies of hybrid zones within *Sceloporus* are no exception (Arévalo *et al*. 1993; Sites *et al*. 1996). The occurrence of four mtDNA clades in this contact zone is unique, and although the greatest mtDNA divergence (> 10.6%; Appendix I) is between *S. cowlesi* and *S. tristichus*, most of the morphological and karyotypic divergence is found within *S. tristichus*. If phenotypic and karyotypic divergence were coupled with mtDNA divergence in these lizards, we would expect to find the greatest morphological and chromosomal disparity between *S. cowlesi* and *S. tristichus*. Thus, this contact zone contains multiple processes operating simultaneously, including hybridization between morphologically and karyotypically diagnosable mtDNA clades within *S. tristichus*, and secondary contact between two distantly related yet morphologically cryptic mtDNA lineages (*S. tristichus* and *S. cowlesi*).

The presence of hybrid lizards with heteromorphic pairs of chromosomes and apparently successful meiosis in heterozygotes indicates that reproductive isolation is not complete among some or all clades of lizards interacting in the hybrid zone at different localities. Estimates of cytonuclear disequilibria in the centre of the zone demonstrate that the northern and southern clades of *S. tristichus* are in equilibrium with respect to the nuclear alleles (Table 2), which implies that these subclades are not reproductively isolated. But what conclusions can we draw regarding hybridization between *S. cowlesi* and *S. tristichus*? *Sceloporus cowlesi* haplotypes also enter the hybrid zone, but they are

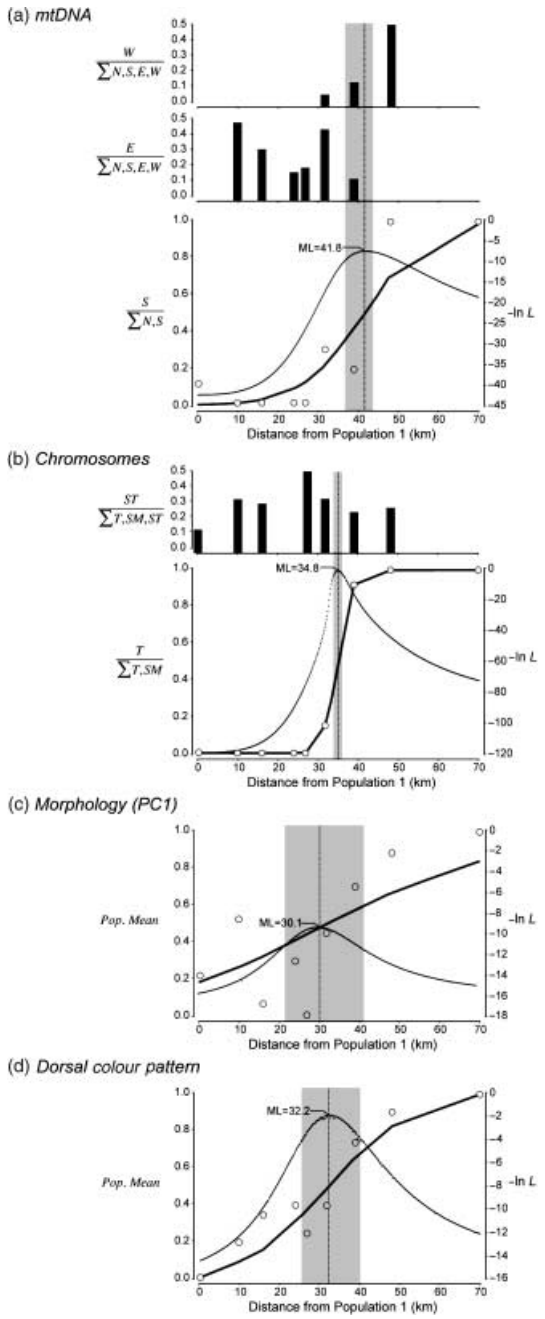

Fig. 8 Changes in the frequency of (a) mtDNA haplotypes (b) chromosomes (c) morphology, and (d) dorsal colour patterns across the Silver Creek transect between the grassland (northern) and woodland (southern) populations of *Sceloporus tristichus* and *Sceloporus cowlesi*. Locality number one corresponds to Holbrook, the most northerly locality. Bold curves are tanh clines fitted to population means (circles) using maximum likelihood. Dotted lines are likelihood profiles for cline centres (right vertical axes). The vertical dashed lines indicate the maximum-likelihood estimate of the cline centre, and the grey boxes correspond to two log-likelihood support limits (values presented in Table 3). Frequency histograms for the western and eastern haplotypes and the subtelocentric chromosome are shown above the mtDNA and chromosome clines, respectively.

not pervasive and their exclusion from the southern portion of the transect suggests that *S. cowlesi* does not interact equally with the northern and southern subclades of *S. tristichus*. Only *S. cowlesi* haplotypes show a significant nonrandom association with nuclear alleles (Table 3), suggesting that *S. cowlesi* and at least one of the *S. tristichus* subclades are not randomly mating or that their offspring experience epistatic effects on fitness (Asmussen *et al*. 1987). *Sceloporus cowlesi* and the southern *S. tristichus* clade are sympatric to the exclusion of other *S. tristichus* clades ~20 mi to the east of the Silver Creek transect at localities #13 and #16 (Fig. 6), and this separate contact zone provides an opportunity to determine if the interactions among the various clades are indeed different.

Formation of the hybrid zone

Most hybrid zones are found at ecotones between two distinct habitats (Barton & Hewitt 1985), and the hybrid zone discussed here is no different. Differentiated subclades belonging to *S. tristichus* hybridize in the Little Colorado River basin along canyon ecotones between Great Basin Conifer Woodland and Great Basin Grassland communities. It is intriguing that the recent history of this landscape includes drastic vegetative alterations as a result of cattle overgrazing dating from the late 1800s (Abruzzi 1995). The maximum-likelihood cline-fitting analyses suggest that the centre of the *S. tristichus* mtDNA cline is significantly different from the other clines and appears to be shifted by as much as 8.5 km to the south (Fig. 8, Table 3). A southward shift in the mtDNA cline is consistent with the hypothesis of a northward-moving hybrid zone with mtDNA introgression, where selectively neutral mtDNA haplotypes are left in the 'wake' of the moving hybrid zone (Parsons *et al*. 1993; Rohwer *et al*. 2001). Our separate temporal analyses of the karytoype data provide some support for this hypothesis and suggest that the contact zone is moving north by as much as 0.5 km per decade (Table 3). If the mtDNA cline centre marks the location of the origin of the contact zone, then only 170 years were required for the contact zone to reach its 2002 position (8.5 km at 0.5 km per 10 years). These figures for the rate of movement of the cline are conservative because our estimate of the cline centre for the 1970s includes the 2002 data, which are skewing the estimates towards the current-day position. Thus, the cline may be proceeding north at a faster rate than we can provide with the current data. The northward spread of juniper trees into the former grassland environment after the onset of cattle grazing could have promoted the increased dispersal of southern *S. tristichus* populations into the hybrid zone. This scenario provides a mechanism for the shift of the hybrid zone to the north and the resulting lack of coincidence in the character clines. However, we recognize that a similar ecotone may have existed in the general area

for a long period of time, shifting in location concomitant with long-term climatic changes. Without a historic sample of specimens collected from the region prior to the onset of cattle grazing it may be impossible to test whether the hybrid zone has a recent origin, or if the timing of the influx of *S. cowlesi* and the various *S. tristichus* clades was the same. Indeed, it is also possible that some of the observed variants in the mtDNA and chromosomes evolved in the area of the hybrid zone.

The *S. undulatus* complex exhibits remarkable levels of geographical variation in all character systems examined, which is a prerequisite for the formation of a complex hybrid zone like the one described here. These high levels of variation are not exclusive to the southwestern USA, but extend across much of North America (Cole 1972; Leaché & Reeder 2002; Miles *et al*. 2002). We believe that additional contact zones will be discovered in the *undulatus* complex as other species boundaries are scrutinized more closely. We cannot predict if these contact zones will demonstrate similar levels of complexity as exhibited in the *S. cowlesi* + *S. tristichus* contact zone discussed here, but it is nonetheless important to determine if hybridization is a common phenomenon in this group of lizards.

Species limits in the Sceloporus undulatus *complex*

The possibility that our mtDNA data do not portray accurately the species history cannot be ignored (Funk & Omland, 2003). Discordance between mtDNA gene trees and species trees can have multiple sources, including incomplete lineage sorting of ancestral polymorphisms, sex-biased life histories, and introgression (Harrison 1991; Avise 1994, 2000). The presence of sex-biased home ranges and dispersal in *S. 'undulatus'* (*sensu lato*) could also generate discrepancies between mtDNA gene trees vs. nuclear markers. Females have substantially smaller home ranges compared to males (Ferner 1974; Jones & Droge 1980; Haenel *et al*. 2003), which could result in increased levels of mtDNA substructure that are not evident in the nuclear genome. Under the scenario of a sex-biased life history, we may not expect nuclear loci to support the fine-scale phylogeographical structure provided by mtDNA. If future analyses of nuclear DNA data (currently underway) conflict with the existing mtDNA genealogy, then the current phylogenetic and taxonomic framework for the *undulatus* complex will require re-evaluation.

The *S. undulatus* complex and the contact zone studied here provide a clear example of the contentious intersection between interpretation of empirical data and species delimitation. A central issue in the species concept debate, which extends into differences among species delimitation methods, is disagreement over the most important criteria for diagnosing species. Criteria can be divided into those that emphasize pattern (e.g. monophyly) vs. those that recognize process (e.g. evolution of reproductive isolation). Both categories have advantages, but no single criterion is likely to produce biologically meaningful results when applied rigidly across all cases (O'hara 1993; Mayden 1997; de Queiroz 1998; Pigliucci 2003; Wake 2006). While we believe that *S. 'undulatus'* (*sensu lato*) represents more than one species, we acknowledge that any inference of the number of species in this complex is directly linked to the particular 'threshold' one imposes to define species status (Agapow *et al*. 2004; de Queiroz 2005). With respect to the hybrid zone studied here, it is data conflict, and not confusion regarding species concepts, that obscures the number of evolutionary lineages in the *undulatus* complex. Morphologically distinct populations are nested within the same species, while the underlying genetic divergence suggests the presence of two highly differentiated, yet morphologically cryptic lineages. Despite the challenges imposed by this apparent decoupling of morphological, karyotypic, and mtDNA divergence, detailed analyses of contact zones such as the one studied here are necessary to determine the evolutionary processes responsible for speciation in the *undulatus* complex.

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Adam Leaché is a PhD student studying evolutionary biology, phylogenetics, and population genetics. His dissertation research is focused on deciphering species limits in fence lizards. Dr Charles J. Cole's research for decades has focused on herpetological population genetics, hybrid zones, systematics, and the evolutionary biology of unisexual species of hybrid origins.

Appendix I

Locality data for *Sceloporus cowlesi* and *Sceloporus tristichus* samples included in the mitochondrial DNA gene tree. The data are arranged by mtDNA clade, beginning with contact zone localities containing sympatric haplotypes belonging to *S. cowlesi* and *S. tristichus*, followed by the *S. cowlesi* and *S. tristichus* clades. Localities with haplotypes nested within the single *S. cowlesi* clade or three *S. tristichus* clades found in the contact zone, but which do not contain mixed haplotypes, are named according to their orientation with respect to the hybrid zone (see Fig. 3). The maximum uncorrected sequence divergence (uncorrected 'p' distances) is provided for localities with a sample size ≥ two individuals

Locality no.	Specific locality	Latitude	Longitude	Sample size	Maximum sequence divergence
Sympatric haplotype localities					
1	AZ; Navajo, 0.5 mi N Holbrook	34.92006	–110.15642	4	0.04231
2	AZ; Navajo, 0.5 mi S Holbrook	34.90933	–110.152	4	0.05057
3	AZ; Navajo, 5 mi. E Old Woodruff Rd.	34.86022	–110.00281	2	0.10217
4	AZ; Navajo, Fivemile Wash	34.83582	–110.1449	15	0.10320
5	AZ; Navajo, Washboard Wash	34.79292	–110.09873	14	0.10526
6	AZ; Navajo, 3 mi. S Woodruff	34.73806	–110.03756	6	0.10320
7	AZ; Navajo, 5 mi S Woodruff	34.71233	–110.03322	5	0.10423
8	AZ; Navajo, 4 mi NE Dry Lake	34.68172	–110.34969	14	0.10114
9	AZ; Navajo, 11.2 mi. N Snowflake (Hwy 77)	34.66950	–110.10883	4	0.10217
10	AZ; Navajo, Silver Creek, 13 mi N Snowflake	34.66770	–110.045	43	0.10630
11	AZ; Navajo, Silver Creek, 9.3 mi N Snowflake	34.62820	–110.05563	3	0.10217
12	AZ; Navajo, Silver Creek, 7 mi N Snowflake	34.60250	–110.0624	13	0.10423
13	AZ; Apache, 10 mi W Concho	34.53218	–109.76963	19	0.10114
14	AZ; Navajo, Snowflake	34.52789	–110.08072	10	0.04850
15	AZ; Navajo, 11.8 mi N Show Low	34.42080	–110.08900	5	0.04954
***S. cowlesi* mtDNA Lineage**					
Eastern contact zone clade					
16	AZ; Apache, 2 mi E Hunt	34.61928	–109.66219	12	0.00206
17	AZ; Apache, 2 mi SW Saint Johns	34.47932	–109.38483	2	0.00000
18	AZ; Apache, 20 mi S Sanders	35.00660	–109.22950	2	0.00000
19	AZ; Apache, 6 mi E Springerville	34.15472	–109.21883	2	0.00000
20	NM; McKinley, 4 mi N McGaffey	35.46713	–108.55302	2	0.00000
21	NM; McKinley, Twin Buttes Rd.	35.45737	–108.83198	1	—
Sister clades					
22	AZ; Cochise, Willcox Playa	32.25000	–109.83330	2	0.00000
23	AZ; Pinal, Oracle	32.61470	–110.7907	1	—
24	AZ; Santa Cruz, 5 mi SE Elgin	31.61610	–110.48330	1	—
25	NM; Bernalillo, Cedro	35.01950	–106.34883	1	—
26	NM; Dona Ana, 25 mi W El Paso	31.91970	–106.94910	1	—
27	NM; Eddy, Whites City	32.17710	–104.38020	1	—
28	NM; Grant, 4 mi N Pinos Altos	32.90000	–108.23900	3	0.00929
29	NM; Guadalupe, 6 mi N Santa Rosa	35.03570	–104.68910	2	0.00206
30	NM; Guadalupe, Vegas Junction	34.97873	–104.99065	1	—
31	NM; Hidalgo, San Simon Valley	31.92070	–109.00277	1	—
32	NM; Lincoln, 5 mi W Carrizozo	33.68330	–105.93550	1	—
33	NM; Otero, White Sands N.M.	32.79970	–106.26030	1	—
34	NM; San Miguel, 5 mi S Los Montoyas	35.33247	–105.14952	2	0.00103
35	NM; San Miguel, Villanueva	35.25933	–105.36498	1	—
36	NM; Sandoval, Cibola N.F.	35.28268	–106.47843	2	0.00103
37	NM; Socorro, 6 mi NW Magdelena	34.23770	–107.36430	1	—
38	NM; Socorro, Magdelena Mtns	34.02122	–107.10626	2	0.00000
39	NM; Socorro, San Antonio	33.91720	–106.87390	1	—
40	NM; Torrance, Manzano	34.66800	–106.32290	1	—
41	TX; Brewster, 8 mi S Alpine	30.28070	–103.58550	1	—
42	MX; Chihuahau, 7 mi S Samalayuca	31.35000	–106.46667	1	—
43	MX; Chihuahua, Rancho El Milagro	31.10000	–107.98333	1	—

Appendix I *Continued*

Locality no.	Specific locality	Latitude	Longitude	Sample size	Maximum sequence divergence
***S. tristichus* mtDNA Lineage**					
Northern contact zone clade					
44	AZ; Apache, Teec Nos Pos	36.91840	–109.08920	1	—
45	AZ; Navajo, 2 mi W Holbrook	34.90481	–110.19444	5	0.00206
46	UT; San Juan, 14.5 mi SW Mexican Hat	37.05633	–110.08817	1	—
Western contact zone clade					
47	AZ; Navajo, 3.5mi E Winslow	35.00540	–110.65310	5	0.00206
48	AZ; Navajo, Clear Creek Reservoir	34.96867	–110.64505	6	0.04747
Southern contact zone clade					
49	AZ; Gila, 55.1 mi N Globe, HWY 77	33.95683	–110.33217	3	0.00619
50	AZ; Navajo, 4 mi SW Show Low	34.21833	–110.10817	11	0.00619
51	AZ; Navajo, 4 mi W Shumway	34.41331	–110.11756	1	—
52	AZ; Navajo, 5 mi NW Show Low	34.33528	–110.10339	12	0.00413
53	AZ; Navajo, 6 mi SW Taylor	34.40062	–110.11768	12	0.00413
Sister clades					
54	AZ; Coconino, 1 mi S Grand Canyon	36.00017	–111.79800	1	—
55	AZ; Coconino, 10 mi S Tusayan	35.93583	–112.12000	2	0.00103
56	AZ; Coconino, 17 mi N Valle	35.80250	–112.13083	1	—
57	AZ; Coconino, 3 mi NW Bitter Springs	36.67000	–111.61950	1	—
58	AZ; Coconino, 8 mi S TenX campground	35.85500	–112.13383	1	—
59	AZ; Coconino, Angel Rd.	35.19300	–111.38078	4	0.04850
60	AZ; Coconino, Angel Rd.	35.16378	–111.28635	1	—
61	AZ; Coconino, Kaibab Lake	35.28290	–112.15222	2	0.00103
62	AZ; Coconino, Marshal Lake	35.11900	–111.53990	5	0.00206
63	AZ; Coconino, Willaha	35.76217	–112.26400	2	0.00206
64	AZ; Coconino, Willard Springs	34.97190	–111.68380	3	0.00310
65	AZ; Coconino, Wupatki	35.48920	–111.25350	3	0.00310
66	AZ; Gila, 23.3 mi. E Payson, HWY 260	34.30333	–110.99017	6	0.05263
67	AZ; Gila, 27.8 mi N Globe, HWY 77	33.68833	–110.57217	4	0.02167
68	AZ; Gila, Canyon Creek	34.24360	–110.80110	3	0.00516
69	AZ; Gila, Sixshooter Canyon	33.31150	–110.79500	1	—
70	AZ; Mojave, Music Mountains	35.74783	–113.85000	1	—
71	AZ; Mojave, Music Mountains	35.73667	–113.81000	1	—
72	AZ; Mojave, Willows Ranch Rd.	35.20050	–113.43050	4	0.00413
73	AZ; Navajo, 1.5 mi NW Heber	34.44300	–110.61917	5	0.00103
74	AZ; Pinal, Oak Flat Rec. Area	33.30810	–111.05013	5	0.00206
75	AZ; Yavapai, Yarnell	34.21850	–112.74883	1	—
76	CO; Costilla, Rio Grande River	37.18062	–105.72867	2	0.00103
77	CO; La Plata, 2 mi S Durango	37.24790	–107.89092	1	—
78	CO; Mesa, 3 mi SW Whitewater	38.95983	–108.47450	4	0.00929
79	CO; Montezuma, Yellow Jacket Canyon	37.52000	–108.70122	3	0.00000
80	CO; Montrose, 5 mi N Naturita	38.30350	–108.66733	3	0.00000
81	NM; San Juan, Blanco	36.72383	–107.83550	2	0.00310
82	NM; San Juan, Newcomb	36.30540	–108.69580	1	—
83	NM; San Miguel, 4 mi W Sands	35.42053	–105.55657	1	—
84	NM; Santa Fe, 2 mi W Glorieta	35.57913	–105.78970	1	—
85	NM; Taos, 2 mi E Taos	36.37812	–105.55228	2	0.00000
86	NM; Taos, Tres Piedras	36.65370	–105.96680	3	0.00000
87	UT; Garfield, Henry Mountains	37.83780	–110.61200	1	—
88	UT; Iron, 2 mi E Paragonah	37.90750	–112.74600	5	0.00206
89	UT; Kane, 2 mi NW Kanab	37.08517	–112.51117	1	—
90	UT; San Juan, 20 mi SE Moab	38.42883	–109.42100	1	—
91	UT; Uintah, Book Cliffs	40.01180	–109.71750	2	0.00000
92	UT; Washington, Leeds	37.23860	–113.35830	1	—
93	WY; Sweetwater, 30 mi SW Rock Springs	41.46340	–109.37960	1	—

Appendix II

Chromosome seven morphology and sample sizes at localities sampled through the contact zone. The data are arranged in descending order based on latitude (from north to south), and localities with samples collected in both the 1970s and 2002 are separated into two rows labeled 'a' and 'b', respectively. Chromosome seven centromere position codes are as follows: SM, submetacentric; ST, subtelocentric; and T, telocentric

Locality no.	Specific locality	Latitude	Longitude	Samples (year)	Chromosome 7 morphology (# of samples) homozygotes / heterozygotes
1	AZ; Navajo, 0.5 mi N Holbrook	34.92006	–110.15642	4 (2002)	SM + SM (3) / SM + ST (1)
94	AZ; Navajo, 1 mi WNW Holbrook	34.91720	–110.17780	4 (1976)	SM + SM (4)
2	AZ; Navajo, 0.5 mi S Holbrook	34.90933	–110.15200	2 (2002)	SM + SM (2)
45	AZ; Navajo, 2 mi W Holbrook	34.90481	–110.19444	4 (2002)	SM + SM (3), ST + ST (1)
4a	AZ; Navajo, Fivemile Wash	34.83582	–110.14490	15 (1971, 72, 73, 76)	SM + SM (5), ST + ST (2) / SM + ST (8)
4b	—	—	—	8 (2002)	SM + SM (5) / SM + ST (3)
5	AZ; Navajo, Washboard Wash	34.79292	–110.09873	9 (2002)	SM + SM (5), ST + ST (1) / SM + ST (3)
6a	AZ; Navajo, 3 mi. S Woodruff	34.73806	–110.03756	1 (1975)	SM + SM (1)
6b	—	—	—	1 (2002)	SM + SM (1)
7	AZ; Navajo, 5 mi S Woodruff	34.71233	–110.03322	2 (2002)	SM + SM (1), ST + ST (1)
8	AZ; Navajo, 4 mi NE Dry Lake	34.68172	–110.34969	3 (2002)	T + T (2), ST + ST (1)
10a	AZ; Navajo, Silver Creek, 13 mi N Snowflake	34.66770	–110.04500	54 (1972, 73, 75, 76)	SM + SM (24), ST + ST (11), T + T (1) / SM + ST (9), T + SM (5), T + ST (4)
10b	—	—	—	26 (2002)	SM + SM (14), ST + ST (2) / SM + ST (9), T + SM (1)
95	AZ; Navajo, 9.3 mi N Snowflake (Hwy 77)	34.64416	–110.08833	7 (1972–76)	ST + ST (3), T + T (2) / SM + ST (1), T + SM (1)
16	AZ; Apache, 2 mi E Hunt	34.61928	–109.66219	7 (2002)	ST + ST (2), T + T (1) / T + ST (4)
96	AZ; Navajo, Silver Creek, 7.9 mi N Snowflake	34.60888	–110.06333	15 (1972–76)	T + T (6), SM + SM (1) / T + ST (7), T + SM (1)
12	AZ; Navajo, Silver Creek, 7 mi N Snowflake	34.60250	–110.06240	8 (2002)	T + T (5) / T + SM (3)
97	AZ; Coconino, 16 mi WNW Heber	34.59457	–110.79285	5 (1976)	T + T (5)
13	AZ; Apache, 10 mi W Concho	34.53218	–109.76963	10 (2002)	T + T (10)
14	AZ; Navajo, Snowflake	34.52789	–110.08072	8 (2002)	T + T (5), ST + ST (1) / T + ST (2)
52	AZ; Navajo, 5 mi NW Show Low	34.33528	–110.10339	9 (2002)	T + T (9)
98	AZ; Navajo, Pinedale	34.30750	–110.25150	2 (1972)	T + T (2)
99	AZ; Navajo, 3.5 mi N Show Low	34.30694	–110.03777	5 (1973)	T + T (5)
100	AZ; Navajo, 2.6 mi E Pinedale	34.30190	–110.20080	2 (1973)	T + T (2)
101	AZ; Navajo, 2.8 mi N Show Low	34.29805	–110.03440	6 (1971)	T + T (6)
			Total:	217	SM + SM (69), T + T (61), ST + ST (25) /
				116 (1970s) / 101 (2002)	ST + SM (34), T + ST (17), T + SM (11)

Appendix III

Voucher specimens of *Sceloporus tristichus* and *Sceloporus cowlesi* included in the study. Numbers in bold correspond to localities listed in tables one and two. Localities 102–106 were sampled exclusively for the morphometric study. The types of data collected for each specimen are designated with the letters 'D' (mtDNA), 'K' (karyotype), and 'M' (morphometrics). Standard museum abbreviations follow Leviton *et al.* (1985). Non-standard and personal field series abbreviations are as follows: LVT, University of Nevada Las Vegas Tissue Collection; TK, Texas Tech University Tissue Collection; ADL, Adam D. Leachè; TWR, Tod W. Reeder; LEB, Lars E. Bell; JQR, Jon Q. Richmond

1: *AMNH 153949-52 (D,K,M);* ***2:*** *AMNH 153953-4 (D,K,M), TWR 711-12 (D);* ***3:*** *AMNH 153926-7 (D,M);* ***4:*** *ADL 218 (D), AMNH 154014, 154016, 154024-25 (D,M), AMNH 154015, 154017-23 (D,K,M), TWR 667-8 (D), AMNH 108124-5, 109146, 109148-50, 111101, 111103, 111106, 114115-7, 114119-20 (K,M), 111104 (K,M), 109147, 109151-2, 111102, 111105, 114118, 114121 (M);* ***5:*** *ADL 220 (D), AMNH 153968-70, 153972-77 (D,K,M), AMNH 153971, 153978-80 (D,M);* ***6:*** *AMNH 153928 (D,K,M), AMNH 154009-13 (D,M), AMNH 112494 (K,M);* ***7:*** *AMNH 153929-30 (D,K,M), AMNH 153994-6 (D,M);* ***8:*** *AMNH 153931-7, 153939, 153942-43, 154037 (D,M), AMNH 153938, 153940-1 (D,K,M);* ***9:*** *TWR 707-10 (D);* ***10:*** *AMNH 153892-4, 153896-03, 153905-7, 153909, 153912-7, 153920, 153922-5 (D,M,K), AMNH 153895, 153904, 153908, 153910-11, 153918-9, 153921 (D,M), ADL 223, 225-6, SDSU 4163-8 (D); AMNH 109137-40, 109143, 109159, 111107-11, 111115-20, 111122-26, 111128, 111130-32, 112479-82, 112485-93, 114125-26, 114129-34, 114136-7 (K,M), AMNH 111113, 111121, 111127, 111129, 114127 (K), AMNH 109136, 109141, 109144, 109158, 111114, 111134-5, 112483-4, 112489, 114135, 114139 (M);* ***11:*** *ADL 227-9 (D);* ***12:*** *AMNH 153981-88 (D,K,M), AMNH 153989-93 (D,M);* ***13:*** *AMNH 154038-40, 154045-50, 154064 (D,K,M), ADL 230-1 (D), AMNH 154041-44, 154063, 154065-6 (D,M);* ***14:*** *AMNH 154026-28, 154030, 154032-34 (D,K,M), AMNH 154029, 154035-6 (D,M), 154031 (K,M);* ***15:*** *TWR 617, 720-23 (D);* ***16:*** *AMNH 154052-3, 154057-61 (D,K,M), AMNH 154051, 154054-56, 154062 (D,M);* ***17:*** *ADL 232-3 (D);* ***18:*** *ADL 234-235 (D);* ***19:*** *LEB 185-6 (D);* ***20:*** *ADL 237-8 (D);* ***21:*** *ADL 236 (D);* ***22:*** *TWR 928-9 (D);* ***23:*** *TWR 1062 (D);* ***24:*** *ADL 103 (D);* ***25:*** *SDSU 4252 (D);* ***26:*** *TWR 380 (D);* ***27:*** *LVT 00362 (D);* ***28:*** *TWR 521-3 (D);* ***29:*** *ADL 250-1 (D);* ***30:*** *ADL 252 (D);* ***31:*** *LSUMZ 448817 (D);* ***32:*** *ADL 55 (D);* ***33:*** *SDSU 4218 (D);* ***34:*** *ADL 255 (D);* ***35:*** *ADL 249 (D);* ***36:*** *ADL 243-4 (D);* ***37:*** *LEB 187 (D);* ***38:*** *SDSU 4110, JQR 162 (D);* ***39:*** *TK 24286 (D);* ***40:*** *SDSU 4247 (D);* ***41:*** *TWR 947 (D);* ***42:*** *UIMNH 36351 (D);* ***43:*** *UIMNH 36314 (D);* ***44:*** *LVT 00706 (D);* ***45:*** *AMNH 153945-8 (D,K,M), AMNH 153944 (D,M);* ***46:*** *TWR 1754 (D);* ***47:*** *ADL 157-161 (D);* ***48:*** *ADL 212-7 (D);* ***49:*** *TWR 671-3 (D);* ***50:*** *TWR 662, 685-91, 715-7 (D);* ***51:*** *AMNH 153955 (D,M);* ***52:*** *AMNH 153956-9, 153962-3, 153965-7 (D,K,M), 153960-1, 153964 (D,M);* ***53:*** *ADL 30-41 (D);* ***54:*** *TWR 2030 (D);* ***55:*** *TWR 2022-3 (D);* ***56:*** *TWR 2028 (D);* ***57:*** *TWR 1755 (D);* ***58:*** *TWR 2029 (D);* ***59:*** *ADL 204-7 (D);* ***60:*** *ADL 209 (D);* ***61:*** *LVT 02287, ADL 201 (D);* ***62:*** *SDSU 4158-62 (D);* ***63:*** *TWR 2025-6 (D);* ***64:*** *ADL 129-32 (D);* ***65:*** *SDSU 4234-6 (D);* ***66:*** *TWR 698-703 (D);* ***67:*** *TWR 618, 678-80 (D);* ***68:*** *UTA 50699-701 (D);* ***69:*** *TWR 1895 (D);* ***70:*** *TWR 2017 (D);* ***71:*** *TWR 2020 (D);* ***72:*** *TWR 1665-7, 2013(D);* ***73:*** *TWR 693-7 (D);* ***74:*** *SDSU 4229-33 (D);* ***75:*** *SDSU 4237 (D);* ***76:*** *ADL 271-2 (D);* ***77:*** *ADL 273 (D);* ***78:*** *TWR 1822-5 (D);* ***79:*** *ADL 275-7 (D);* ***80:*** *ADL 280, TWR 1858-9 (D);* ***81:*** *TWR 1875-6 (D);* ***82:*** *LVT 02305 (D);* ***83:*** *ADL 247 (D);* ***84:*** *ADL 246 (D);* ***85:*** *ADL 263-4 (D);* ***86:*** *ADL 265-7 (D);* ***87:*** *UTA 50772 (D);* ***88:*** *TWR 1690-3, 1605 (D);* ***89:*** *TWR 1725 (D);* ***90:*** *TWR 1786 (D);* ***91:*** *BYU 45982, 45984 (D);* ***92:*** *TK 24222 (D);* ***93:*** *ADL 189 (D);* ***94:*** *AMNH 114109, 114111 (K,M), AMNH 114112-3 (K), AMNH 114108, 114110 (M);* ***95:*** *AMNH 109153-6, 112495-6, 114124 (K,M);* ***96:*** *AMNH 109160, 109162-3, 109165-6, 111091, 111095, 112476-8, 114144, 114146 (K,M), AMNH 111090, 111092, 111094 (K), AMNH 109161, 109164, 109167-9, 114141-3, 114145 (M);* ***97:*** *AMNH 114103-7 (K);* ***98:*** *AMNH 108928-9 (K,M);* ***99:*** *AMNH 112611-5 (K,M);* ***100:*** *AMNH 112620-1 (K,M);* ***101:*** *AMNH 108074-7, 108079, 108081 (K,M), AMNH 108078, 108080 (M);* ***102:*** *AMNH 112616-9 (M);* ***103:*** *AMNH 114046-7, 114055, 114059-60, 114064-7, 114069 (M);* ***104:*** *AMNH 114524 (M);* ***105:*** *AMNH 109145 (M);* ***106:*** *AMNH 109157 (M).*

Appendix IV

Descriptive statistics of the morphological characters with all samples pooled. Data for males (top) and females (bottom) are presented for each character. Character abbreviations are as follows: SVL, snout-vent-length; HW, head width; HL, head length; TL, length of the fourth toe; DS, number of dorsal scales; LAM, number of lamellae on fourth toes; FEMS, number of femoral pores; THAR, throat patch area; ABAR, abdominal patch area. Descriptions of each character are provided in the methods section

	SVL (mm)	HW (mm)	HL (mm)	TL (mm)	DS (count)	LAM (count)	FEMS (count)	THAR (pixels)	ABAR (pixels)
Minimum	40	7.86	9.79	7.30	36	34	25	97	896
	40	7.82	9.20	7.65	37	35	24	20	647
Maximum	80	15.64	16.52	14.42	48	51	40	2812	13788
	87	16.29	17.23	14.61	49	49	40	1439	11496
Median	67	12.93	14.21	12.12	42	43	32	841.5	7274.0
	70	13.07	14.22	12.07	42	42	32	415	5129.5
Mean	64.4	12.67	13.77	11.76	42.0	42.5	32.4	896.1	7084.2
	66.1	12.55	13.64	11.57	42.3	42.0	31.7	436.1	5113.1
Standard deviation	9.4	1.73	1.65	1.57	2.5	3.1	2.6	465.0	3063.9
	12.5	2.09	1.95	1.68	2.6	3.0	2.7	251.7	2637.9
Variance	88.5	3.00	2.71	2.48	6.2	9.8	6.9	216193.2	9387436.9
	156.3	4.36	3.82	2.83	6.8	9.1	7.4	63335.2	6958629.2