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Divergent character clines across a recent secondary contact zone in a Hispaniolan lizard

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Abstract

Studies of genetic contact zones provide valuable information regarding the processes of population divergence, adaptation and speciation. In this paper, I examine transitions in morphology, mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) haplotypes across a recent secondary contact zone in a Hispaniolan lizard *Ameiva chrysolaema*. Maximum likelihood cline fitting analyses suggest non-coincidence of cline centers and that the mtDNA cline is significantly displaced to the west of the remaining clines. nDNA and morphological clines are coincident and tend to be associated with the prevailing environmental gradient. The lack of cytonuclear disequilibrium near the center of the contact zone and the non-coincidence of character clines suggest that this zone does not conform to a tension zone model of hybridization; thus, gene flow across the zone does not seem to be impeded. The extremely narrow width of the dorsal scale size cline and the close association of this cline with the steepness of the environmental (precipitation) gradient suggest that this character may be under environmental selection. Taken together, this contact zone appears to be structured by a combination of mtDNA introgression, possibly associated with eastward movement of the zone, and environmental selection on some characters.

Introduction

Environmental transition zones (i.e. ecotones) are important in structuring biodiversity (Endler, 1977; Smith *et al*., 1997; Schneider *et al*., 1999; Moritz *et al*., 2000) and in defining the range limits of species (Case & Taper, 2000; Cicero, 2004). These zones are also important for conservation as they represent areas of high species turnover (i.e. Spector, 2002). Frequently, ecotones are associated with areas where divergent lineages or species make secondary contact. During allopatric episodes, population divergence results in character differences between populations, thus generating character clines when populations make secondary contact (Endler, 1977). Patterns of character transitions across contact zones provide information about the nature and consequences of hybridization (Endler, 1977; Barton & Hewitt, 1985; Harrison, 1993; Arnold, 1997). The extent and patterning of gene exchange across a contact zone can be described by the relative strengths of environment-independent (endogenous) and environment-dependent (exogenous) selection (Barton & Hewitt, 1985; Arnold, 1997). Endogenous selection refers to selection against hybrid genotypes. In this case the zone is maintained by balance between selection against hybrid genotypes and dispersal of parental genotypes and is referred to a tension zone (Barton & Hewitt, 1985). Exogenous selection refers to differential natural selection across an environmental gradient (Endler, 1977; Moore & Price, 1993).

Discriminating between endogenous and exogenous selection is difficult, because both modes of selection result in similarly shaped clines. However, certain characteristics of character clines can provide useful information to aid in discrimination (Moore & Price, 1993). If exogenous selection structures the contact zone, the position and width of a cline should be determined by the position and width of the ecotone. Where the ecotone is narrow and steep, the character cline should be narrow and steep; where the ecotone is gradual, the character cline should be shallow. Zones structured by endogenous selection tend to be environment independent. Furthermore, selection against hybrids in these zones should result in the spatial clustering of different character clines. Recent studies have found evidence of both endogenous and exogenous selection in a variety of different hybrid zones. For example, in both salamanders (Alexandrino *et al*., 2005) and chickadees (Bronson, Grubb & Braun, 2003), strong selection against hybrid genotypes implies that endogenous selection structures these hybrid zones. Alternatively, the strong association of character clines with environmental variation in crickets (Ross & Harrison, 2002) and some bird species imply the operation of exogenous selection (quail Gee, 2004; titmice Cicero, 2004).

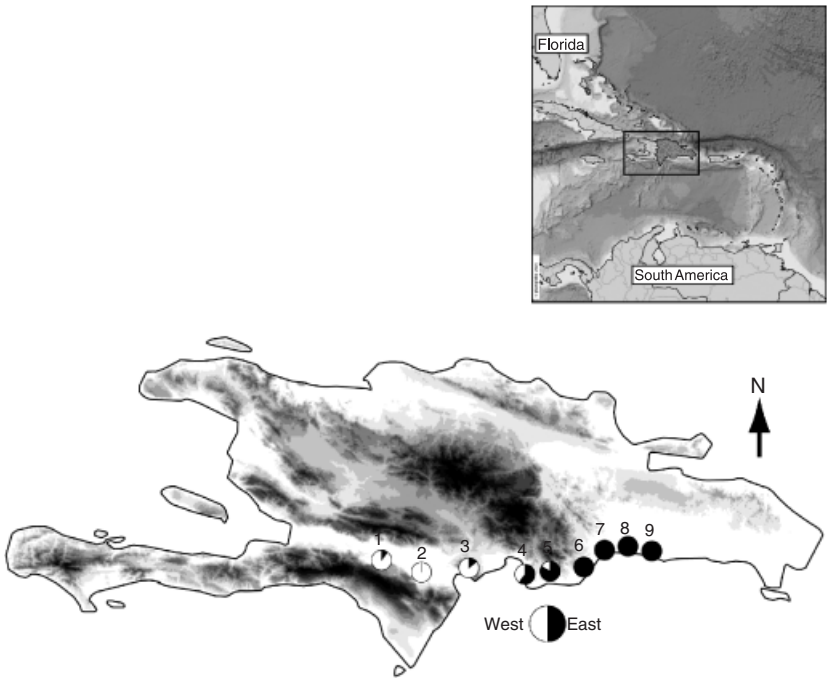

Figure 1 Map of Hispaniola denoting sampling locations used in this study. Pie charts represent the proportion of mitochondrial DNA haplotypes at each site deriving from eastern (black) and western (white) lineages.

This study examines transitions in morphology and DNA [mitochondrial DNA (mtDNA) and nuclear DNA (nDNA)] haplotypes across a secondary contact zone between divergent mtDNA lineages of a ground-dwelling lizard in the Dominican Republic. The goals are as follows. First, I test whether the mtDNA lineages differ in morphology. Second, I examine the clinal patterns of genetic and morphological variation to characterize the contact zone. Last, I examine the coincidence and condordance of character clines and their relationships to environmental variation across the zone to assess the processes responsible for cline structure.

Ameiva chrysolaema and the ecology of the contact zone

This contact zone is located near an environmental transition zone along the southern coast of Hispaniola in the Dominican Republic (Fig. 1). The mountainous topography of the island and the prevailing northern trade-winds result in a steep gradient of precipitation over *c*. 25 km. This steep gradient plays an important role in structuring Hispaniolan biodiversity as it marks the range limits of several lizard species (Schwartz & Henderson, 1991; Gifford, 2005) and other faunal elements (Powell, Ottenwalder & Inchaustgui, 1999). *Ameiva chrysolaema* is a large ground-dwelling teiid lizard endemic to Hispaniola. This species is widely distributed in lowland habitats and, unlike many other Hispaniolan lizard species, is distributed across the ecotone. Schwartz & Henderson (1991) originally noted the contact zone as a zone of intergradation between two subspecies of *A. chrysolaema* (*Ameiva chrysolaema boekeri* and *Ameiva chrysolaema procax*). Recent genetic evidence confirms the location of this zone (Gifford *et al*., 2004; Gifford & Larson, unpubl. data), although subspecific designations are not supported.

Differentiating between primary intergradation and secondary contact is notoriously difficult (Endler, 1977; Barton & Hewitt, 1985; Willett, Ford & Harrison, 1997). However, phylogeographic analysis of genetic variation has been useful for discrimination between these alternatives (Harrison, 1993; Willett *et al*., 1997). The genetic structure within *A. chrysolaema* indicates a history of allopatric fragmentation followed by recent secondary contact of lineages near the ecotonal boundary (Gifford *et al*., 2004; Gifford and Larson, unpubl. data). This fragmentation event is estimated to be *c*. 1 million years old (kya) and is associated with a period of sea inundation of lowland areas of Hispaniola (Gifford & Larson, unpubl. data) [Correction added after publication 19 October 2007: in the preceding sentence, 'This fragmentation event is estimated to be *c*. 400 000 years old' was corrected to 'This fragmentation event is estimated to be *c*. 1 million years old']. This zone of secondary contact provides the opportunity to study the interaction between selection and gene flow and the relationships of these processes to population divergence.

Materials and methods

Transect location and sampling

I sampled an *c*. 200-km longitudinal transect spanning the mtDNA contact zone and the environmental transition zone. This transect is located in the south-central Dominican Republic and extends eastward along the southern coast. In order to increase sample size, I combined localities within 10–20 km of one another into a single sample. This

scheme resulted in nine sampling locations along the transect (Fig. 1). Sampling for genetic analysis consisted of an average of 13 individuals per location (range = 5–26) and derive from Gifford *et al.* (2004) and Gifford & Larson (unpubl. data). Museum specimens from the same or nearby localities were obtained to augment sampling for morphological analyses. Morphology sampling averaged 23 individuals per location (range = 7–35).

Morphological data

I measured nine linear morphological traits on each of 210 specimens to the nearest 0.01 mm using digital calipers (Mitutoyo; a list of specimens examined is included in an online appendix). Measurements included the following: snout–vent length (SVL), measured from the tip of the snout to the anterior tip of the cloaca; head length (HL), measured from tip of the snout to the anterior margin of the auricular opening; head width (HW), measured at the widest point; head height (HH), measured at the tallest point on the head; body length (BL), measured as the distance between the insertion of the fore- and hind-limbs; femur length (FL), measured from the insertion of the hind limb to the knee; tibia length (TL), measured from the knee to the base of the wrist; metatarsus length (ML), measured from the base of the wrist to the insertion of the longest toe; and toe length (TOEL), measured from the insertion of the longest toe to the tip excluding the claw. I also included a measure of scale size because studies have suggested that this character may be associated with aridity in lizards (Soule & Kerfoot, 1972; Thorpe & Baez, 1987, 1993; Malhotra & Thorpe, 1997; Calsbeek, Knouft & Smith, 2006). Scale size is inversely correlated with scale number; hence as a proxy for scale size, I counted the number of scales in a fixed area on the dorsal surface of each specimen using digital photographs. I photographed the dorsal surface of each specimen from a fixed focal distance using a Canon EOS 30D digital SLR equipped with a 100-mm macro lens and ring flash (Canon USA Inc. Lake Success, NY, USA). A 2.5-mm^2 grid was included in each photograph for scale. A 2.5-mm^2 box was drawn in Adobe Photoshop 5.5 and placed over the mid-dorsal surface of each specimen and the number of scales falling within this area was counted. Although unconventional, counts from digital photographs were preferable because the dorsal scales of teiid lizards are very small and granular, making accurate counting difficult (Table 1).

Genetic data

mtDNA data are from the ND2 protein-coding gene (Gifford *et al.*, 2004; Gifford & Larson, unpubl. data). In these studies we identified two divergent groups of haplotypes that diagnose eastern and western lineages. For this study, these haplotypes are collapsed into alternative 'alleles' representing the eastern and western groups. nDNA data derive from a length polymorphism (5 bp indel) identified in the α-enolase intron 8 locus (E8; Gifford & Larson, unpubl. data). This polymorphism is easily scored from sequence electropherograms and shows clinal variation across the contact zone but alternative alleles (presence or absence of the 5 bp deletion) are not fixed on opposite sides of the transect (save locality #9 on the eastern side).

Quantification of environmental variation

I quantified environmental variation along the transect by first plotting individual localities in DIVA GIS version 5.4 (Hijmans *et al.*, 2005*b*). For each locality I extracted three composite environmental variables (BIOCLIM) relating to temperature and precipitation compiled from the WORLDCLIM vers. 1.4 dataset (Hijmans *et al.*, 2005*a*). These variables represent *c.* 10-year global climate surfaces at a resolution of 1 km^2. The three composite variables included BIO1 (mean annual temperature), BIO12 (mean annual precipitation) and BIO17 (precipitation during the driest quarter) and were chosen because they were deemed important for xeric lizard physiology.

Data analysis

All linear morphological measurements were $\log_{10}$ transformed before analysis to meet the assumption of normality. I tested for sexual dimorphism in linear measurements with ANCOVA using $\log_{10}$SVL as a covariate. All traits showed significant sexual dimorphism (results not shown); hence males and females are analyzed separately. Only results for male lizards are reported; females showed qualitatively similar results. All linear measurements were significantly correlated with body size (SVL); hence they were first regressed against SVL and residuals were computed. Residuals were then used in canonical variates analysis (CVA). I used CVA to test whether it is possible to objectively differentiate populations into eastern and western groups. Scale data were analyzed separately. Scale data were square-root transformed because they represented an area across the lizard's dorsum. This transformation resulted in a linear relationship between square-root-adjusted scale counts and $\log_{10}$SVL ($R^2 = 0.572$, $F_{1,131} = 172.08$, $P < 0.0001$). ANCOVA indicates no sexual dimorphism in dorsal scale counts; thus male and female data were combined for analysis. Environmental differences between geographic regions were also examined using one-way ANOVA with region (east vs. west) as the fixed effect.

For examination of cline shape, I plotted mean CV1 scores (males and females independently), mean residuals from regression of square-root-transformed scale counts on $\log_{10}$SVL (males and females combined), $\log_{10}$ transformed environmental variables, E8 allele frequency and mtDNA haplotype frequency for each locality against distance (km) along the transect. Morphological and environmental data were scaled from 0 to 1 to represent western and eastern populations, respectively. I fitted tanh curves through the DNA and morphological cline data using the 'Fit 1D Cline' operation in the program ANALYSE version 1.3 (Barton & Baird, 1999). I computed model parameters using 200 iterations from 20 different starting points. I estimated the two-likelihood support limits for each parameter (i.e. cline center and width) using the 'cross-section' option in

Table 1 Summary of linear morphometric and scale count data for each sample locality

Locality	*n*	SVL	HL	HW	HH	BL	FL	TL	ML	TOEL	Scales (*n*)[a]
Males											
1	19	104.52 ± 1.69	24.86 ± 0.38	15.78 ± 0.32	14.32 ± 0.23	49.13 ± 0.86	19.19 ± 0.26	21.72 ± 0.38	14.40 ± 0.25	21.71 ± 0.45	65.04 ± 2.15 (27)
2	22	103.13 ± 1.99	24.83 ± 0.49	15.99 ± 0.49	14.62 ± 0.36	49.20 ± 1.04	19.41 ± 0.34	21.22 ± 0.39	14.20 ± 0.21	21.34 ± 0.35	68.20 ± 2.43 (35)
3	8	94.64 ± 5.02	22.28 ± 1.15	13.61 ± 0.97	12.61 ± 0.91	44.24 ± 2.71	17.03 ± 0.99	19.30 ± 1.06	12.97 ± 0.71	20.76 ± 0.75	75.18 ± 6.67 (11)
4	17	96.22 ± 2.18	23.21 ± 0.49	13.67 ± 0.45	13.07 ± 0.37	43.01 ± 1.27	17.83 ± 0.38	19.98 ± 0.42	13.58 ± 0.21	20.98 ± 0.30	76.56 ± 3.24 (25)
5	19	107.86 ± 2.63	25.47 ± 0.71	15.52 ± 0.55	14.73 ± 0.52	51.09 ± 1.41	19.51 ± 0.50	22.31 ± 0.62	14.85 ± 0.30	23.34 ± 0.53	65.17 ± 3.30 (29)
6	19	106.51 ± 3.44	25.18 ± 0.82	15.71 ± 0.67	14.24 ± 0.55	48.25 ± 1.84	19.55 ± 0.64	22.19 ± 0.17	14.78 ± 0.36	22.08 ± 0.49	75.11 ± 4.89 (28)
7	7	114.22 ± 4.25	25.96 ± 0.98	15.76 ± 0.85	14.54 ± 0.58	51.66 ± 2.66	19.89 ± 0.97	23.04 ± 0.89	14.71 ± 0.48	22.87 ± 0.48	75.09 ± 5.53 (11)
8	4	115.94 ± 6.61	26.76 ± 1.50	16.04 ± 1.50	14.98 ± 1.28	53.03 ± 3.89	21.45 ± 1.31	23.85 ± 1.23	15.73 ± 0.68	24.48 ± 0.63	77.67 ± 9.51 (6)
9	19	113.27 ± 3.64	26.89 ± 0.85	15.85 ± 0.69	15.34 ± 0.58	54.37 ± 2.19	21.31 ± 0.70	23.95 ± 0.77	16.23 ± 0.43	25.74 ± 0.55	72.41 ± 4.62 (27)
Females											
1	10	94.76 ± 2.09	21.93 ± 0.34	12.58 ± 0.26	11.96 ± 0.24	44.86 ± 1.26	17.11 ± 0.55	19.43 ± 0.36	13.14 ± 0.20	19.90 ± 0.48	
2	13	91.78 ± 1.40	21.28 ± 0.27	12.30 ± 0.19	11.82 ± 0.19	44.82 ± 1.28	16.89 ± 0.22	18.29 ± 0.25	12.32 ± 0.16	18.12 ± 0.27	
3	4	92.39 ± 4.85	20.91 ± 1.21	12.03 ± 0.84	11.53 ± 0.60	41.80 ± 2.80	16.25 ± 1.04	18.70 ± 1.20	12.15 ± 0.78	19.55 ± 0.78	
4	13	85.79 ± 2.02	20.22 ± 0.48	10.99 ± 0.26	11.11 ± 0.45	41.22 ± 1.14	15.56 ± 0.34	17.63 ± 0.25	11.70 ± 0.15	17.87 ± 0.28	
5	11	91.88 ± 2.79	20.82 ± 0.62	11.83 ± 0.33	11.47 ± 0.38	43.03 ± 1.82	16.76 ± 0.53	19.01 ± 0.57	12.54 ± 0.34	19.55 ± 0.51	
6	10	94.31 ± 4.17	21.32 ± 0.72	12.05 ± 0.47	11.39 ± 0.50	44.46 ± 2.51	16.98 ± 0.70	19.40 ± 0.85	12.93 ± 0.45	19.53 ± 0.50	
7	4	107.85 ± 2.30	23.71 ± 0.56	13.18 ± 0.28	12.48 ± 0.32	49.65 ± 1.23	18.25 ± 0.58	21.48 ± 0.62	13.90 ± 0.27	20.90 ± 0.45	
8	3	104.42 ± 4.22	23.12 ± 1.07	12.98 ± 0.52	12.10 ± 0.46	48.00 ± 3.96	19.33 ± 0.72	20.70 ± 0.49	13.93 ± 0.47	21.57 ± 0.75	
9	8	96.85 ± 3.82	22.13 ± 0.75	11.91 ± 0.43	11.76 ± 0.40	46.41 ± 2.02	18.65 ± 0.54	20.25 ± 0.60	13.64 ± 0.37	21.61 ± 0.45	

[a]Scale data represent male and female lizards combined.

Table entries are mean ± 1 standard error. All linear measurements are in millimeters.

SVL, snout–vent length; HL, head length; HW, head width; HH, head height; BL, body length; FL, femur length; TL, tibia length; ML, metatarsus; TOEL, toe length.

ANALYSE. I tested the concordance of cline centers using a maximum likelihood approach similar to that used by Brumfield *et al.* (2001). For each dataset, I estimated the model log-likelihood with the cline center fixed at 10 km intervals while allowing cline width to vary freely. Fixed cline centers span the range of observed cline centers estimated from all three datasets. A log-likelihood ratio test (LRT) was used to compare a model that assumed cline coincidence with an unconstrained model estimated from each datasets. The test statistic was calculated as two times the absolute difference in log-likelihood between the constrained and unconstrained models. Significance of the LRT was determined by comparison to a χ^2 table ($\alpha = 0.05$) with degrees of freedom (d.f.) equal to the difference in the number of parameters estimated in the models. I also tested whether character clines are coincident with the prevailing precipitation gradient using an LRT. I estimated the center of the precipitation cline by noting the place at which the cline crosses a frequency of 0.5. I tested coincidence at 1 km intervals, over a range of 20 km (130–150 km). Significance was tested the same as above. Contact zones structured by selection against hybrids often find evidence for non-random associations between nuclear alleles and mtDNA haplotypes near the center of the zone. I tested for cytonuclear disequilibrium (D_M^A) near the center of the zone using the software program CNDd (Asmussen & Basten, 1996; Basten & Asmussen, 1997). For this analysis I pooled localites #4, #5 and #6 to increase sample size.

Results

Morphological and environmental variation between regions

The CVA on linear morphology data suggests that lizards from the eastern and western regions can be readily diagnosed on the basis of morphological features (Fig. 2). These results indicate that lizards in the west have relatively more robust heads (longer, wider and taller) and relatively shorter feet (metatarsus and TOEL) than lizards in the east. Lizards from localities in the contact zone tend to have intermediate CV1 scores (Fig. 2). For scale counts, lizards from the east have significantly more dorsal scales per unit area than lizards from the west (ANCOVA, covariate = $\log_{10}$ SVL, $F_{1,117} = 77.02$, $P < 0.0001$, no significant interaction effect SVL × region). All three environmental variables differed significantly between eastern and western regions (ANOVA; BIO1, $F_{1,6} = 23.82$, $P = 0.008$; BIO12, $F_{1,6} = 32.81$, $P = 0.005$; BIO17, $F_{1,6} = 59.66$, $P = 0.002$), indicating that localities in the west have higher temperatures and lower precipitation than localities in the east.

Cline shape analyses and cytonuclear disequilibrium

Environmental clines all show a steep transition along the transect. The cline for BIO1 shows an inverse relationship to the clines for BIO12 and BIO17, which are nearly identical

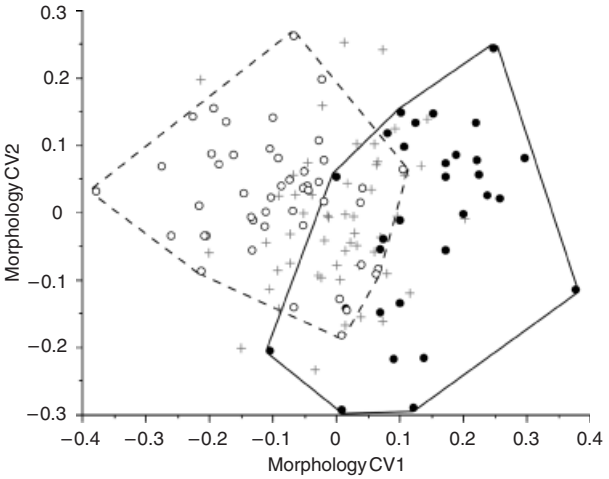

Figure 2 Plot of composite variables generated from the canonical variates analysis on linear morphometric traits. Western (open circles, localities # 1, # 2, # 3), central (gray crosses, localities # 4, # 5, # 6) and eastern (filled circles, # 7, # 8, # 9) groups of populations are shown. Male data shown; results from female data were qualitatively similar.

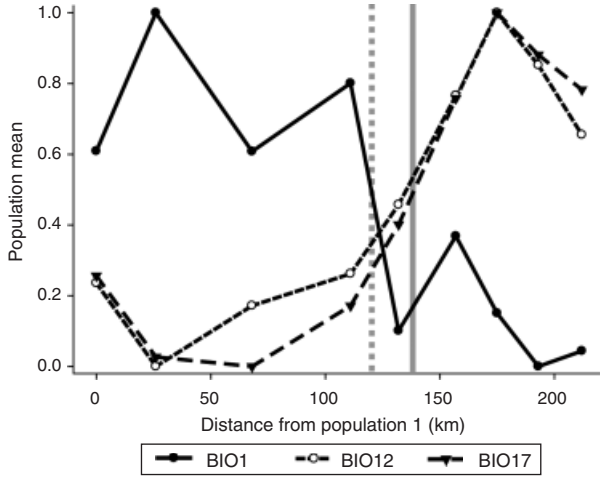

Figure 3 Clines of environmental data across the sampled transect. The *x*-axis represents distance (km) from locality #1. The *y*-axis is the mean value of each environmental variable scaled between 0 and 1. Solid and dashed vertical gray lines denote the approximate centers for the precipitation (BIO12 and BIO17) and temperature (BIO1) clines, respectively.

to one another (Fig. 3). The estimated centers of these clines (location at which the cline equals 0.5) vary slightly. The approximate center of the BIO1 cline is about midway between locality #4 and locality #5 (~120 km from locality #1). The centers for the two precipitation clines (BIO12 and BIO17) are located just east of locality #5 (~135 km from locality #1).

Estimated cline centers are divergent and an LRT strongly rejects the coincidence of nDNA, mtDNA, scale and morphology CV1 clines (Tables 2 and 3). When the mtDNA cline is excluded, the hypothesis of cline

Table 2 Maximum likelihood estimates of cline shape parameters (km)

	mtDNA	E8 indel	Scales	Morphology CV1[a]
Cline center	110.3 (94.3–120.3)	143.9 (127–157)	155.3 (129.7–158.7)	153.4 (136.0–171.0)
Cline width	45.3 (24.3–83.3)	93.9 (57–176)	5.9 (0.0–34.6)	114.3 (69.0–188.0)

[a]Male data only. Female data produced similar results.
Two log-likelihood support limits are shown in parentheses.
mt, mitochondrial DNA.

Table 3 Results of the likelihood ratio test of the null hypothesis that all cline centers are not significantly different from one another

	mtDNA	E8 Indel	Scales	Morphology CV1[a]
$\ln L_u$	−1.35	−2.65	−13.15	−8.63
$\sum = \ln L_u$	−25.78 (−24.43)			
$\sum = \ln L_c$	−47.52 (−25.14)			
$\Delta = [\sum \ln L_c - \sum \ln L_u]$	21.74 (0.71)			
$\chi^2 = 2\Delta$	**43.48** (1.42)			

[a]Male data only. Female data produced similar results.
$\ln L_u$ is the unconstrained log-likelihood support. $\ln L_c$ is the maximum constrained log-likelihood in an analysis where the cline center was fixed at 10 km intervals across the range of individual cline center estimates while all other cline parameters were free to vary. Values in parentheses represent the test when the mtDNA cline is excluded from analysis. Critical χ^2 values are 7.815 (d.f. = 3) and 5.991 (d.f. = 2) for the analysis including and excluding the mtDNA cline, respectively. Bold table entries indicate rejection of the null hypothesis at $\alpha = 0.05$.
mt, mitochondrial DNA.

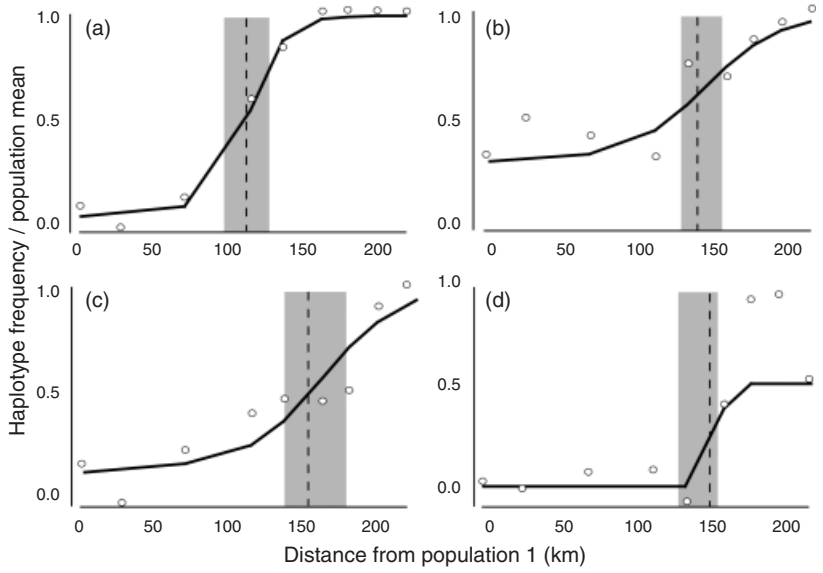

Figure 4 Changes in frequency of (a) mitochondrial DNA haplotypes, (b) α-enolase intron 8 indel polymorphisms, (c) morphology CV1 values and (d) relative number of dorsal scales [from regression of scale number against body size (snout vent length)]. Curves are tanh clines fitted to population means (circles) using maximum likelihood. The vertical dashed lines represent the maximum likelihood estimates of the cline centers, and the gray boxes encompass the two log-likelihood support limits (from Table 2).

coincidence between the remaining three clines cannot be rejected (Table 3). The cline center for mtDNA is shifted significantly to the west (*c.* 35–45 km) of the other clines (Table 2, Fig. 4). The maximum likelihood estimate of the width of the scale cline indicates a very steep transition across the transect (Table 2). Tests of coincidence between character and environmental clines indicate that the nDNA and morphology clines together are broadly compatible with the precipitation cline center estimates (centers = 136–150, all $G < 4.867$, d.f. = 2, $P > 0.05$). On the other hand, the hypothesis of cline coincidence can be rejected for all tested environmental cline centers and mtDNA (centers = 130–150, $P < 0.05$). There was no significant evidence for a non-random association between nuclear and mitochondrial alleles near the center of the contact zone (Fisher's exact test, all $P > 0.143$), suggesting free gene exchange within the zone.

Discussion

Previous phylogeographic studies identified a secondary contact zone between divergent mtDNA lineages of *A. chrysolaema* located along the southern coast of the Dominican Republic (Gifford *et al.*, 2004; Gifford & Larson,

unpubl. data). Like most contact zones, this one is located near a steep environmental transition zone (Barton & Hewitt, 1985). This ecotone has formed as a consequence of the extreme topography of Hispaniola and prevailing northern trade winds. Thus, the major environmental features characterizing this ecotone are precipitation and temperature. Western localities are extremely hot and dry while eastern localities have more moderate temperatures with higher levels of rainfall throughout the year. I used maximum likelihood cline fitting analyses to examine trait variation across this contact zone and to evaluate factors maintaining cline shapes.

Eastern and Western mtDNA lineages are diagnosable on the basis of morphology CV1 (head dimensions and foot length) and scale size. Both these characters, nDNA allele frequency and mtDNA haplotype frequency, show clinal variation across the contact zone. There is strong evidence that all four clines are not coincident; the mtDNA cline center (near locality #4) is located *c*. 35–45 km to the west of the remaining cline centers (near locality #6).

Formation of the contact zone

The contact zone is geographically located at the southern apex of the Cordillera Central (Fig. 1), the major northwest–south-east-running mountain range on Hispaniola. Episodic inundation of Hispaniola likely caused this mountain range to serve as a significant vicariant barrier separating eastern and western mtDNA lineages during the Pleistocene (Gifford *et al*., 2004; Gifford & Larson, unpubl. data). Amelioration of this barrier to gene flow allowed eastern and western lineages to contact one another. The absence of cytonuclear disequilibrium suggests a lack of selection against hybrid genotypes at the center of the zone. This, along with the significant westward displacement of the mtDNA cline, suggests that this contact zone does not conform to a tension zone model. The non-coincidence of clines is consistent with the hypothesis of mtDNA introgression associated with eastward movement of the contact zone, toward the ecotone (Parsons, Olson & Braun, 1993; Rowher, Bermingham & Wood, 2001; Leache & Cole, 2006). The current elevation of the eastern and western regions and the history of seawater inundation provide some support for this interpretation. Much of the current distribution of western populations lies below sea level in the Valle de Neiba. As such, during inundations more time was likely necessary for habitat to become available when sea levels receded (McLaughlin, van den Bold & Mann, 1991). Conversely, the eastern region was likely emergent at an earlier time, owing to its greater elevational relief, providing suitable habitat for range expansion following recession of seawater. Given this information, the origin of the contact zone was likely located near the center of the mtDNA cline. A Multilocus estimate of gene flow between the eastern and western regions is high and mostly unidirectional towards the west (Gifford & Larson, unpubl. data), exceeding values thought to promote allopatric divergence (Slatkin, 1987).

Exogenous selection and morphology

Although endogenous and exogenous selection often result in similar cline shapes (Barton & Hewitt, 1985), certain characteristics of the clines may help to discriminate between these processes for cline maintenance (Moore & Price, 1993). Two lines of evidence suggest that exogenous selection may play a role in structuring variation in the *A. chrysolaema* contact zone. First, endogenous selection often results in the spatial clustering of different clines because of genome-wide selection against hybrid genotypes (Barton & Hewitt, 1985). In this study, I showed that mtDNA and morphology clines are geographically non-coincident and thus are not spatially clustered. Second, exogenous selection predicts that the position and width of the character clines will closely match the position and width of the ecotone. The position of the scale cline is coincident with the estimated position of the precipitation clines. The widths of the precipitation clines are also relatively narrow (*c*. 10–20 km). Similarly, the width of the scale cline is very narrow and spans only about 6 km. This narrow width suggests that selection may be playing a strong role in structuring the scale cline independent of other morphological features (i.e. morphology CV1). Further evidence for environmental selection is provided when phenotypic characters shift spatially in association with a spatial environmental shift. At the eastern end of the transect, precipitation steadily drops from a maximum at locality #7 to lower values at localities #8 and #9. Accordingly, scale counts show a similar pattern of variation, closely following the change in precipitation (Figs 3 and 4). A number of studies have examined patterns of scalation in lizards with respect to environmental variation (Soule & Kerfoot, 1972; Thorpe & Baez, 1987, 1993; Malhotra & Thorpe, 1997; Calsbeek *et al*., 2006). My results are consistent with the findings of these studies in that lizards in more xeric environments tend to have larger scales (i.e. relatively fewer scales) than lizards in more mesic environments. The genetic basis of scale size has not been examined in *Ameiva*, although Calsbeek *et al*. (2006) suggest that the scale characters examined in their study of *Anolis* lizards do have moderate heritability estimates and thus can respond to selection. The broad width (*c*. 114 km) of the morphology CV1 cline suggests that selection on these characters is weak, clinally varying, or neutral. Further data, in addition to those presented here, are necessary to discriminate among these alternatives.

Conclusions

Genetic and morphological variation across a recent secondary contact zone in *A. chrysolaema* reveals a complex interplay between neutral processes and environmental selection. Non-coincident and non-concordant clines suggest that the characters examined respond independently to different forces in the contact zone. These results suggest that although populations appear to be merging via neutral diffusion, the scale size remains highly divergent and strongly associated with the prevailing precipitation

gradient. Additional genetic markers are necessary to evaluate the robustness of these results; however, I tentatively suggest that the *A. chrysolaema* contact zone is structured by eastward movement of the zone associated with mtDNA introgression and environmental selection on dorsal scale characters, consistent with the hypothesis of phenotypic divergence despite gene flow. Unlike other lizard species on Hispaniola whose ranges are sharply demarcated by the precipitation gradient examined here (Schwartz & Henderson, 1991), the environmental gradient does not seem to be a formidable barrier to neutral gene exchange in *A. chrysolaema* but appears to structure phenotypic variation across this zone.

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