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A comparison of nuclear and mitochondrial cline shapes in a hybrid zone in the *Sceloporus grammicus* complex (Squamata; Phrynosomatidae)

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Abstract

The F5 and FM2 chromosome races of the *Sceloporus grammicus* complex form a hybrid zone in the Mexican state of Hidalgo. Previous studies of this zone have assessed genetic structure by averaging estimates of shape and width across three diagnostic chromosome markers. This approach is likely to mask subtle differences in cline shape among loci (e.g. selected vs. neutral), and obscure any displacement of cline centres (if present). Here we use maximum likelihood methods to construct the best fitting individual clines for three chromosomal markers, and also add two new markers; the mitochondrial DNA (mtDNA) locus, and the nuclear ribosomal DNA (rDNA) repeat. For each locus, hybrid zone models were fitted by cline shape and width, and the position and number of segments describing the centre of the zone. Pairwise comparisons between all clines revealed concordance between chromosomes 2 and 6, but significant discordance in cline structure among all other paired combinations. The concordance of chromosomes 2 and 6 suggests that these clines are maintained by genome-wide forces. The discordance of the chromosome 1 cline suggests an influence of asymmetric introgression, while the mtDNA cline is probably influenced by selection and drift. The rDNA locus reveals a pattern best explained by either extreme asymmetric introgression or gene conversion. The structure of zone indicates that genome-wide processes and locus specific selective forces as well as drift, are operating to different degrees on different loci. The locus-by-locus approach used here permits a finer discrimination among possible mechanisms responsible for the maintenance of the individual clines.

Keywords: chromosomes, hybrid zone, mitochondrial DNA, multilocus clines, ribosomal DNA, *Sceloporus grammicus*

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Introduction

Studies of animal hybrid zones have increased dramatically in recent decades. The expanded interest has correlated with deeper theoretical developments in studying population structure and speciation potential (Kruuk *et al*. 1999; for example) as well as advances made in molecular techniques (Hillis *et al*. 1996). Hybridization is now seen as a common phenomenon in nature (Arnold 1997), and one area of research emphasis has been the detailed study of the genetic structure of hybrid zones. Understanding the structure of a hybrid zone provides insights into the evolutionary processes that contribute to its origin and maintenance (Harrison 1993). For example, gene flow, selection, genetic drift and recombination can be estimated with the use of molecular markers. Also, the joint use of cytoplasmic and nuclear markers provides a powerful means for dissecting various sexual asymmetries frequently evident in hybrid zones (Avise 1994).

One system that has provided a wealth of information on vertebrate hybrid zones has been lizards of the *Sceloporus grammicus* complex in Mexico (Squamata; Phrynosomatidae). This complex is made up of at least eight distinct chromosomal races (Arévalo *et al*. 1991), with diploid numbers ranging from $2n = 32$ to $2n = 46$ (Sites & Davis 1989; Arévalo *et al*. 1994). Distribution studies have located a number of hybrid zones between different combinations of these

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races (Arévalo *et al*. 1991), and several of these have been studied in some detail (Hall & Selander 1973; Arévalo *et al*. 1993; Sites *et al*. 1993).

The single hybrid zone in this complex that has been most extensively studied is located in the Mexican state of Hidalgo, north-west of Mexico City. This zone, known as the Tulancingo transect (Sites *et al*. 1993), is a secondary contact between phylogenetically distantly related races (Arévalo *et al*. 1994). The FM2 race ($2n = 45–46$) is confined to xeric vegetation along the western part of the transect, while the F5 race ($2n = 34$) is associated mostly with oak forest on the eastern side. Because of the large chromosomal differences between these hybridizing populations, and their accessibility, this zone has been the focus of detailed studies of microgeographic genetic structure (Sites *et al*. 1995, 1996), selected fitness correlates in hybrid genotypes (Reed *et al*. 1995a; b; Reed & Sites 1995), and the extent of fluctuating asymmetry evident from ongoing hybridization and back-crossing (Dosselman *et al*. 1998).

The study of Sites *et al*. (1995) used maximum likelihood (ML) methods to estimate the average shape and width of this zone on the basis of the distribution of genotypes of three diagnostic chromosomal markers (autosomal pairs 1, 2 and 6). The three diagnostic chromosomal clines showed largely concordant and coincident shape across the zone, but these parameters were only qualitatively assessed (Sites *et al*. 1995). Sites *et al*. (1995) concluded that natural selection was contributing to maintaining the zone's structure primarily by reduced female fecundity of F_1 genotypes, meiotic malassortment at chromosome 2 in F_1 males (endogenous selection against hybrids), exogenous selection favouring different genotypes in different habitats and dispersal of parental genotypes into the zone.

A subsequent study by Sites *et al*. (1996) estimated the cytonuclear structure of the Tulancingo zone [using the three chromosomal markers and diagnostic mitochondrial DNA (mtDNA) haplotypes] to infer forces likely to be responsible for the maintenance of its genetic structure. This study showed that the three chromosomal markers have broadly concordant cytonuclear disequilibria (with the mtDNA locus) within each subsample. This pattern suggests that the structure of the zone is predominantly a result of deterministic genome-wide forces rather than of forces specific to individual loci or chromosomes. Fitting a nested series of likelihood models to the data showed that assortative mating was unlikely to be maintaining the structure of the zone when compared with the alternative of random mating. This left open the question of the role of selection in the maintenance of the zone (none of the then-available likelihood models incorporated the effects of selection).

The results of Sites *et al*. (1995) and Sites *et al*. (1996) are in agreement that migration of parental genotypes into the zone is a predominant force shaping its genetic structure, while random mating holds at the scale of local breeding units. These results are compelling to the extent that different statistical approaches yielded the same result. Sites *et al*. (1995) also concluded that strong selection, both endogenous and exogenous, was probably operating on this zone, a conclusion derived in part from a consensus estimate of position and width for all three diagnostic chromosomes. Sites *et al*. (1995) used ML methods to search for optima of the cline centre, and replicate runs were carried out starting each cline from a different position. Cline shapes and widths were also varied, but replicate searches were limited to shapes fit by zone centre lines of 5, 10, 15, 20 and 30 segments in length. For each model, degrees of freedom are set by the number of angles which describe the line segments used to initially fit the cline, plus the width (d.f. = 16 for a cline of 15 segments). Finding the optimum solution for problems with many degrees of freedom is recognized as intrinsically difficult, and results from models fitted by Sites *et al*. (1995) revealed multiple local optima.

A serious limitation of this approach is that zone width may be over-estimated relative to the most strongly selected loci if clines at different loci have different widths, and if they are not pulled together by the effects of selection. The theory of multilocus clines (Barton 1983) predicts that above some threshold level of selection, loci will not behave independently (although this threshold may not be clearly defined until the hybrid zone has reached equilibrium, Baird 1995). In the absence of a locus-by-locus analysis, over-estimating hybrid zone width by averaging across unlinked markers may lead to an underestimate of the strength of deterministic or stochastic forces operating on individual loci (see Barton & Gale 1993 for review).

This study uses ML methods to: (i) estimate clines for the mtDNA locus, and we also add genotypes for nuclear ribosomal DNA (rDNA) repeats; and (ii) extend the findings of Sites *et al*. (1995) by estimating the individual clines for each chromosome in the Tulancingo zone. For all cline-fitting searches we employed a beta-test version of the software package ANALYSE (Barton & Baird 1996), and use MODELTEST (Posada & Crandall 1998) to select the best fitting model (cline) for each marker, using the mapped distribution of genotypes for all five loci collected from the same animals. All clines are then tested for concordance with each other by use of a statistical pair-wise comparison between all combinations. Pairs for which statistically significant discordance cannot be demonstrated are inferred to be shaped by strong deterministic forces operating across the genome, or across large linkage groups, while significantly discordant clines are inferred to be most strongly influenced by locus-specific selective or stochastic forces.

Materials and methods

The general location and structure of the zone was determined from collections made in 1985 and 1986 (Sites *et al*.

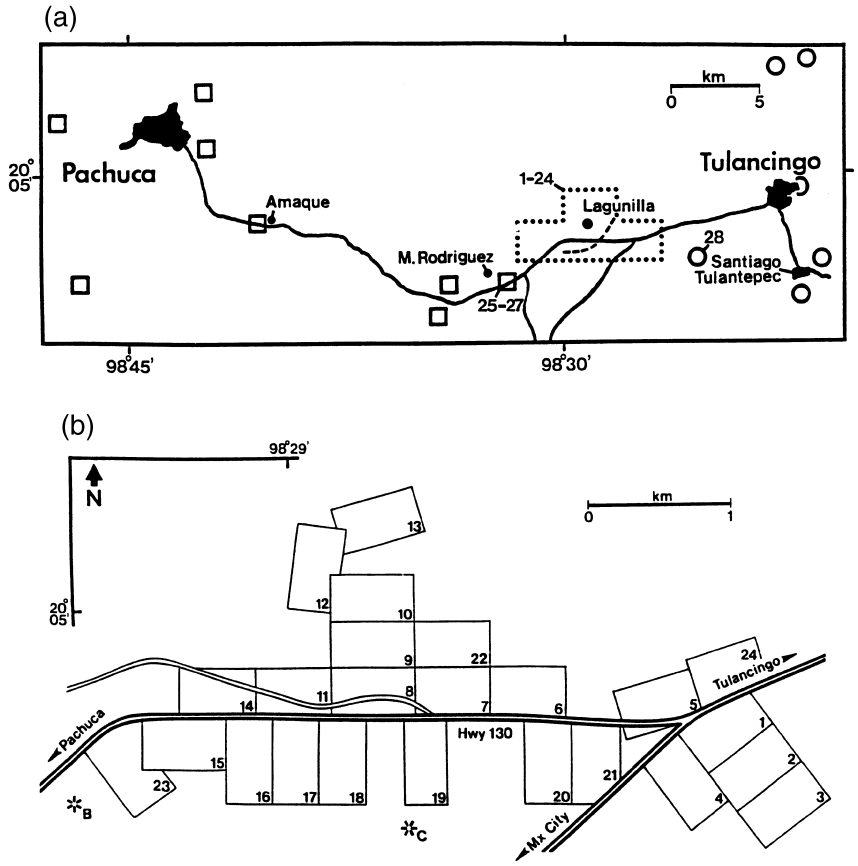

Fig. 1 (a) Map of east-central Hidalgo, Mexico, showing the partial distributions of the *F*5 ($2n = 34$, ○) and FM2 ($2n = 44–46$, □) chromosomal races of the *Sceloporus grammicus* complex. The dotted line delimits the rectangular area mapped in detail during the 1989 and 1991 collections, and includes quadrat maps 1–24 (quadrats 25–27 are outside of this area to the immediate south-west near Matias Rodriguez; quadrat 28 is east-south-east of the main grid area). The dashed line below Lagunilla shows the approximate distribution of chromosomally F_1-like animals in the F5 × FM2 contact region. (b) Positions of the 24 quadrat maps centred on the Tulancingo hybrid zone [dotted grid in (a)], upon which the distributions of lizards were plotted during the two field seasons. Stars at B and C indicate locations of additional animals captured but not plotted on detailed maps, while gaps in the grid (i.e. between quads 18 and 19, or 19 and 20) are areas of cleared pasture devoid of lizard habitat.

1993). In subsequent collections, surveying equipment was used to plot all lizard capture points on 28 individual quadrat maps prepared in the field; all animals were plotted on a scale of 1 : 1000 (Sites *et al*. 1995). Figure 1 shows the general location of the hybrid zone between the towns of Pachuca and Tulancingo, Hidalgo, and the location of the 28 quadrat maps.

A total sample of 502 lizards was taken in the summer of 1989 ($n = 342$) and the spring of 1991 ($n = 160$), and genotypes for chromosomal, mitochondrial and nuclear ribosomal markers were scored from these individuals. Mitochondrial haplotypes were determined by digestion profiles of two polymerase chain reaction (PCR)-amplified target fragments, Eco/ND3 and ND4/Leu, cut with restriction enzymes *Bst*NI and *Hin*fI, respectively, as described by Sites *et al*. (1996). These restriction digests yielded unambiguous diagnostic haplotypes for each chromosomal race (see Fig. 2 in Sites *et al*. 1996), so that all individuals could be scored as either 'F5' or 'FM2'. For the chromosomal markers, specimens were processed for mitotic karyotypes as described by Porter & Sites (1985) and Reed *et al*. (1995a). Rearrangement differences in parental and hybrid karyotypes were coded as nuclear genotypes for chromosomes 1, 2 and 6, as described by Sites *et al*. (1995).

Chromosomes 1 and 6 were unambiguously scored either as AA (bi-armed elements, diagnostic of the F5 race), BB (fissioned elements, diagnostic of FM2), or AB ('hybrid'). Chromosome 2 was diagnostic for each race by two fixed rearrangements, and was therefore more complex, but nevertheless could be unambiguously scored as either AA (bi-armed elements, F5) or B_dB_d ('double fission', diagnostic of FM2). 'F_1-like' genotypes at chromosome 2 were designated AB_d genotypes, but two different chromosomal recombinants were identified, and recombinant genotypes were scored as either AR_c or AR_q (see Fig. 2 in Sites *et al*. 1995).

Nuclear rDNA repeat units were resolved as described by Sites & Davis (1989). Restriction digests were carried out on ~1 μg of DNA in 50 μL reactions, following the manufacturer's specifications (New England Biolabs). Digests were electrophoresed on 0.08% agarose gels, blotted, transferred to filters, hybridized (using a heterologous probe developed for the pocket gopher genus *Geomys*; Davis 1986) and auto-radiographs were developed as described in Hillis & Davis (1986). All restriction sites were mapped using double and partial digests, and restriction sites were coded as present/absent and used to infer the phylogenetic history of all central Mexico races of the *Sceloporus grammicus* complex except F5, which was unknown at the

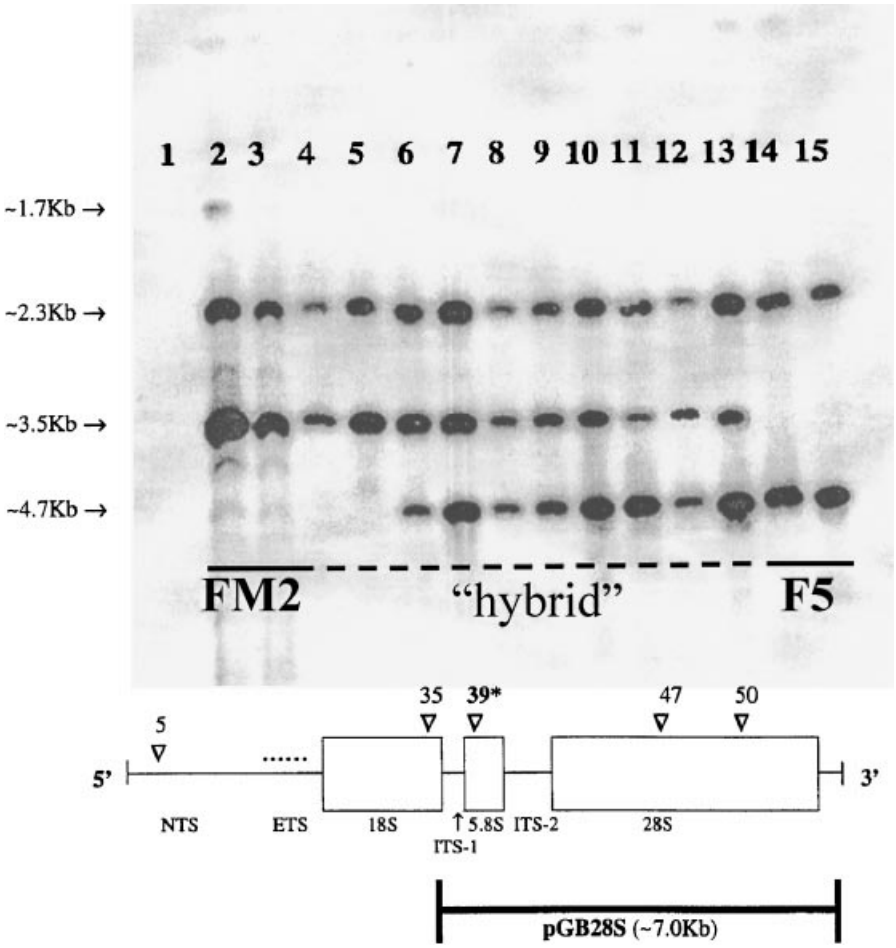

Fig. 2 Autoradiograph of rDNA digestion profile for *Xmn*I in two *Sceloporus grammicus* chromosome races. Lane 1, positive control; lanes 2 and 3, 'pure' FM2 individuals from quad 27 [chromosomal genotypes: $BB/B_dB_d/BB$; $2n = 46$(both female), hybrid index (HI) = 0; voucher numbers IBH 7524–13 and 7524–14; lanes 14 and 15, 'pure' F5 individuals from Tulancingo [genotypes: $AA/AA/AA$; $2n = 33$ (male) and 34 (female); HI = 8; MZFC 4838]; lane 4, 'hybrid' from quad 19 [genotype: $AB/AB_d/AB$; $2n = 39$ (male); HI = 4 (F_1); IBH 7513–2]; lane 5, recombinant from quad 8 [genotypes: $AB/AR_q/AB$; $2n = 38$ (female); HI = 5; IBH 7520–6]; lane 6, $F_1 \times$ F5 backcross from quad 5 [genotype: $AB/AA/AA$; $2n = 35$ (female); HI = 7; IBH 7518–2]; lane 7, F5 from quad 24 [genotype: $AA/AA/AA$; $2n = 34$ (female); HI = 8; IBH 7518–14]; lane 8, F5 from quad 21 [genotype: $AA/AA/AA$; $2n = 34$ (female); HI = 8; IBH 7243]; lane 9, $F_1 \times$ F5 backcross from quad 5 [genotype: $AB/AA/AA$; $2n = 35$ (female); HI = 7; IBH 7518–7]; lane 10, $F_1 \times$ F5 backcross from quad 24 [genotype: $AB/AA/AA$; $2n = 36$ (female); HI = 7; IBH 7518–12]; lane 11, late generation backcross from quad 14 [genotypes: $AA/B_dB_d/AB$; $2n = 40$ (male); HI = 3; IBH 7502–9]; lane 12, F5 from quad 21 [genotype: $AA/AA/AA$; $2n = 33$ (male); HI = 8; IBH 7513–6]; and lane 13, F5 from quad 24 [genotype: $AA/AA/AA$; $2n = 34$ (female); HI = 8; IBH 7518–4]. Below the autoradiograph is a map of the rDNA repeat showing restriction sites (mapped by Sites & Davis 1989). Sites 5, 35, 47 and 50 are present in both races; site 39 is only present in the FM2 race, and the approximate annealing position of the probe pGB28S reveals that cuts at site 35, 47 and 50 would yield fragments of ~4.7, 2.3, and 1.7 kb in the F5 race.

time. Subsequent discovery of the F5 race, and its inclusion in an earlier study of molecular variation across the Tulancingo transect, revealed a diagnostic *Xmn*I restriction site difference between the F5 and FM2 races (Sites *et al*. 1993). Although no further mapping was done, the absence of this restriction site in the 28S gene of the F5 race was consistently evident due to the presence of a large fragment [~4.7 kilobase (kb), Fig. 2] in all 'pure' individuals collected from east of the Tulancingo transect. The presence of the *Xmn*I site in chromosomally pure FM2 individuals from west of the contact was evident in the absence of this large fragment, and the presence of two smaller fragments (the ~3.5 kb fragment and a smaller fragment not seen in Fig. 2) whose approximate sizes (~3.5 + ~1.2 kb) summed to the larger fragment diagnostic of the F5 race (the ~4.7 kb fragment in Fig. 2). Some lizards resolved as 'hybrids' (including many backcross combinations) on the basis of their karyotypes also reflected a 'hybrid' rDNA digestion

profile characterized by the presence of both F5 and FM2 diagnostic fragments (Fig. 2). Scoring for the rDNA locus was therefore given as: F5, FM2, or 'H'. The number of individuals in each group of genotypes scored for all nuclear and mitochondrial loci, for all sampling areas in and adjacent to the Tulancingo zone, is summarized in Appendix I. A complete list of all individual genotypes is available from J.C.M. or J.W.S. upon request.

Mapped capture points from the quads were entered into a computerized grid and all lizards within a radius of 100 m were lumped into single samples (following methods employed by Sites *et al*. 1995). The few samples located outside the plot were included in the analyses but are not included in the figure. A two-dimensional location of the cline centre was fitted by ML using the Metropolis algorithm from the software package ANALYSE (Barton & Baird 1996). For each data set (i.e. each marker) other than rDNA, multiple cline widths and centre line segment numbers were tested. Because the Metropolis algorithm cannot guarantee that the fit of the cline is a universal optimum, it is important that a range of values is tested to increase searches of the cline parameter space. Zone centre segment numbers ranging from 1 to 20 were tested for each marker, and cline widths were varied from 250 to 1500 m based on the optimal range of results reported by Sites *et al*. (1995). Best likelihood scores were then selected for optimal number of segments, and multiple widths (800, 1000 and 1200 m) were run at each of these 'chosen' segment numbers to further refine the fits. More than 100 replicate runs were performed for each marker.

MODELTEST (Posada & Crandall 1998) was then used to compare the best fits by adjusting the ML scores using the Akaike information criteria (AIC). The AIC rewards a model for a good fit but penalizes a model for unnecessary parameters. This ensures that a better fitting parameter-rich model is significantly better than a model with fewer parameters and a slightly worse likelihood score.

Clinal change across the transect was assessed in several ways. First, the best fit clines for each of the markers (C1, C2, C6 and mtDNA) were plotted side by side and compared. Second, ANALYSE (Barton & Baird 1996) was used to visualize concordance of clines by plotting at each site the frequency of each marker against the average frequency of all markers. Finally, we tested for concordance of clines by computing a Bartlett's test for homogeneity for all paired combinations of markers. A χ^2 distribution was used to determine a *P*-value for each test, with Bonferroni adjustments (Hochberg 1988; Rice 1989) across all comparisons. If the *P*-value for a pair of markers was less than 0.05, the null hypothesis of clinal concordance between them was rejected.

Introgression of mtDNA haplotype and rDNA genotype was investigated by plotting per cent of FM2 haplotypes and genotypes present for each of five classes of genotypes found within the hybrid zone. Classifications were based on the chromosome markers C1, C2 and C6, and include: F_1-like individuals, $F_1 \times FM2$ and $F_1 \times F5$ backcross, and pure F5 and FM2 classes. We recognize that many complex matings could occur in this hybrid zone, creating the potential for the production of a large number of distinct classes of genotypes, but with only three diagnostic Mendelian markers, we could not confidently assign any lizard to any genotype class beyond these five. For group 3, the 'F_1-like' genotype, the expected number of mtDNA haplotypes for either race is 50% under random mating/no selection assumptions. We used a small sample exact binomial test to assess the asymmetry of mtDNA in this generation.

Results

For each chromosome and the mtDNA locus, a minimum of 100 searches was performed over a variable number of segments and cline widths (Table 1). First, chromosomes 2 and 6 were consistently fitted to narrower widths (0.8–1.0 km for best scores in chromosome 2, and 0.8 km only for chromosome 6) than chromosome 1 (1.2 km width) and the mtDNA cline (0.9–1.2 km for best likelihood scores). Second, chromosome 2 showed the tightest 'clumping' of best fit models around the narrowest range of segment lengths (13–15 segments) of any locus (Table 1). Chromosome 1 shared an almost equally narrow range of fits (14–17 segments), while chromosomes 6 and mtDNA showed best fits over a wider range of segment lengths (Table 1).

We could not justify arbitrarily selecting one particular cline model for each marker based on best ML score alone, because any particular cline fit might not be statistically better than a cline with one or two fewer segments. We therefore arranged best-fitted clines for each locus in order of increasing segment number, and selected from each the cline with the best AIC score (Table 2). This was necessary to account for the number of parameters required by a particular cline model to get the best ML score, and to penalize more complex models for unnecessary parameters. Table 2 also shows that an increase in number of parameters did not always give a better likelihood score; compare 12-segment with 13-segment models for chromosome 1. Using the AIC criteria, the best fitting models for each of the markers were as follows; the 14-segment model for chromosome 1 (width 1.2 km), the 13-segment model for chromosome 2 (width 0.8 km), the 10-segment model for chromosome 6 (width 0.8 km) and the eight-segment model for the mtDNA (width 1.0 km).

The best fitting models for each of these markers are depicted in Fig. 3. Comparing markers we see that they all have the same general shape, although widths vary from 0.8 to 1.2 km. This is not the case for the rDNA (Fig. 4); here

Table 1 Summary of maximum likelihood cline-fitting searches for four genetic markers scored on *Sceloporus grammicus* from the Tulancingo transect, using the computer program ANALYSE (Barton & Baird 1996)

			Best fitting cline		
Markers	No. of segments	No. of searches	ML score	No. of segments	Cline width
Chr. 1	4,5	11	– 144.812	5	1.25 km
Chr. 1	6–10	27	– 83.090	10	1.25 km
Chr. 1	11,12	16	– 73.656	12	1.20 km
Chr. 1	13	10	– 85.157	13	1.20 km
Chr. 1	14	10	– 71.410	14	1.20 km
Chr. 1	15	11	– 71.411	15	1.20 km
Chr. 1	16	10	– 70.494	16	1.20 km
Chr. 1	17	10	– 70.847	17	1.20 km
Chr. 1	18	10	– 78.379	18	1.20 km
Chr. 1	19	10	– 87.219	19	1.20 km
Chr. 1	20	10	– 92.500	20	1.20 km
Chr. 2	3–8	27	– 98.000	8	0.90 km
Chr. 2	9	16	– 91.377	9	0.80 km
Chr. 2	10	16	– 85.997	10	0.80 km
Chr. 2	11	10	– 89.497	11	0.80 km
Chr. 2	12	10	– 88.724	12	1.00 km
Chr. 2	13	10	– 81.071	13	0.80 km
Chr. 2	14	10	– 81.601	14	0.80 km
Chr. 2	15	10	– 83.406	15	1.00 km
Chr. 2	16	10	– 86.032	16	0.80 km
Chr. 2	17	10	– 87.149	17	0.80 km
Chr. 6	2–20	23	– 67.267	17	0.90 km
Chr. 6	8	5	– 74.665	8	0.80 km
Chr. 6	9	10	– 68.565	9	0.80 km
Chr. 6	10	10	– 65.998	10	0.80 km
Chr. 6	11	10	– 67.367	11	0.80 km
Chr. 6	12	15	– 66.954	12	0.80 km
Chr. 6	13	10	– 69.165	13	0.80 km
Chr. 6	14	10	– 64.113	14	0.80 km
Chr. 6	15	5	– 73.969	15	0.80 km
Chr. 6	16	2	– 74.924	16	0.80 km
Chr. 6	17	10	– 64.898	17	0.80 km
Chr. 6	18	10	– 69.749	18	1.00 km
Chr. 6	19	15	– 62.749	19	0.80 km
mtDNA	2–20	19	– 62.505	11	1.00 km
mtDNA	8	10	– 62.451	8	1.00 km
mtDNA	9	10	– 67.758	9	1.00 km
mtDNA	10	10	– 63.316	10	1.20 km
mtDNA	11	10	– 58.635	11	0.90 km
mtDNA	12	10	– 66.135	12	1.20 km
mtDNA	14	10	– 58.980	14	1.00 km
mtDNA	15	10	– 55.640	15	1.00 km
mtDNA	16	10	– 60.060	16	0.80 km
mtDNA	18	10	– 61.204	18	1.20 km
mtDNA	19	10	– 59.468	19	1.00 km
mtDNA	20	10	– 61.690	20	0.70 km

Scores for only the best fitting clines of each segment number group are given (see text for details). ML score = the negative log maximum likelihood score.

Table 2 A comparison of likelihood scores for cline models of increasing free parameter number, for each of the four genetic markers

Model	Best ML score	Free parameters	AIC score
Chromosome 1			
4 segment	– 711.079	6	1434.158
5 segment	– 144.812	7	303.624
6 segment	– 125.098	8	266.196
7 segment	– 104.342	9	226.684
8 segment	– 105.060	10	230.120
9 segment	– 109.176	11	240.352
10 segment	– 83.090	12	190.180
11 segment	– 100.349	13	226.698
12 segment	– 73.656	14	175.312
13 segment	– 85.157	15	200.314
14 segment	– 71.410	16	**174.820**
15 segment	– 71.411	17	176.822
16 segment	– 70.494	18	176.988
17 segment	– 70.847	19	179.694
18 segment	– 78.379	20	196.758
19 segment	– 87.219	21	216.438
20 segment	– 92.500	22	229.000
Chromosome 2			
3 segment	– 1089.933	5	2189.866
4 segment	– 167.679	6	347.358
5 segment	– 112.680	7	239.360
6 segment	– 103.685	8	223.370
7 segment	– 114.604	9	247.208
8 segment	– 98.000	10	216.000
9 segment	– 91.847	11	205.694
10 segment	– 85.997	12	195.994
11 segment	– 89.497	13	204.994
12 segment	– 88.724	14	205.448
13 segment	– 81.071	15	**192.142**
14 segment	– 81.601	16	195.202
15 segment	– 83.406	17	200.812
16 segment	– 86.032	18	208.064
17 segment	– 87.149	19	212.298
Chromosome 6			
4 segment	– 1456.450	6	2924.900
5 segment	– 113.993	7	241.986
6 segment	– 84.418	8	184.836
7 segment	– 75.970	9	169.940
8 segment	– 74.665	10	169.330
9 segment	– 68.565	11	159.130
10 segment	– 65.998	12	**155.996**
11 segment	– 67.367	13	160.734
12 segment	– 66.954	14	161.908
13 segment	– 69.165	15	168.330
14 segment	– 64.113	16	160.226
15 segment	– 73.969	17	181.938
16 segment	– 74.924	18	185.848
17 segment	– 64.848	19	167.696
18 segment	– 69.749	20	179.498
19 segment	– 62.749	21	167.498
mtDNA			
4 segment	– 181.252	6	374.504
5 segment	– 155.682	7	325.364
6 segment	– 142.772	8	301.544
7 segment	– 72.069	9	162.138
8 segment	– 62.451	10	**144.902**

Table 2 *Continued*

Model	Best ML score	Free parameters	AIC score
9 segment	– 67.758	11	157.516
10 segment	– 63.316	12	150.632
11 segment	– 59.865	13	145.712
12 segment	– 66.135	14	160.270
13 segment	– 70.990	15	171.980
14 segment	– 58.980	16	149.960
15 segment	– 55.640	17	145.280
16 segment	– 60.060	18	156.120
17 segment	– 64.723	19	167.446
18 segment	– 61.204	20	162.408
19 segment	– 59.468	21	160.936
20 segment	– 61.690	22	167.380

Since models were not nested, the Akaike information criterion (AIC) was used for the comparisons, as implemented in the MODELTEST program. The AIC is a useful measure that rewards models for a good fit, but imposes a penalty for unnecessary parameters. The best fitting model for each marker is shown in bold.

the FM2 genotype (the open pie graph) has almost completely introgressed into the F5 race. Only 16% of the chromosomally pure F5 individuals were also pure at the rDNA locus (see Appendix I). The asymmetry of the introgression is so extensive we opted not to include the rDNA repeat in the cline searches; a sufficient number of pure F5 samples was unavailable in this case as external 'anchor points' for inferring a cline shape.

Closer inspection of the best fitting clines suggests that chromosome 2 (Fig. 3b) and chromosome 6 (Fig. 3c) appear to be the most concordant pair, and these are also most similar in width (Table 1) and segment length (Table 2) in the best fitting clines. To visualize better the extent of concordance and discordance among the chromosome and mtDNA clines, we plotted the frequency of each marker against the average frequency of all four markers (Fig. 5). In this case clines are described by cubic polynomials fitted by ML (Szymura & Barton 1986), and all would align on the diagonal if they were perfectly concordant with each other. Figure 5 shows that the fits for chromosome 2 and

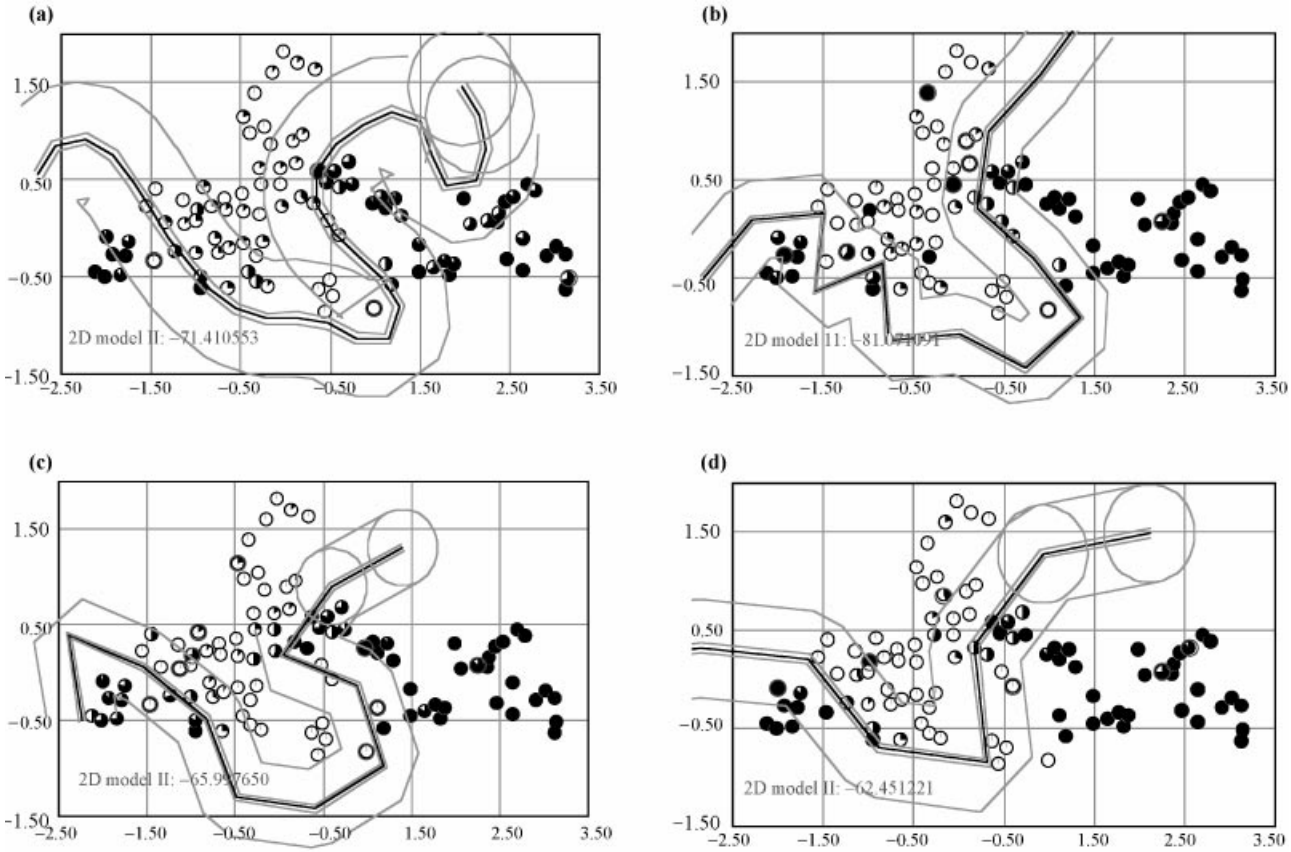

Fig. 3 The best-fitting two-dimensional clines for four of the five genetic markers screened in this study. All clines are superimposed on pie diagrams showing the average frequency of F5 genotypes (solid area) in each sample, and each pie represents a sample formed by pooling lizards closer than 100 m (= 114 sites). The heavy centre line is the fitted cline, while lighter lines on either side show contours half a cline width from the centre. (a) The cline fitted for chromosome 1; ML score = – 71.410, segment number = 14, cline width = 1.2 km. (b) The cline fitted for chromosome 2; ML score = – 81.071, segment number = 13, cline width = 0.80 km. (c) The cline fitted for chromosome 6; ML score = – 65.998, segment number = 10, cline width = 0.80 km. (d) The cline fitted for mtDNA; ML score = – 62.451, segment number = 8, cline width = 1.0 km.

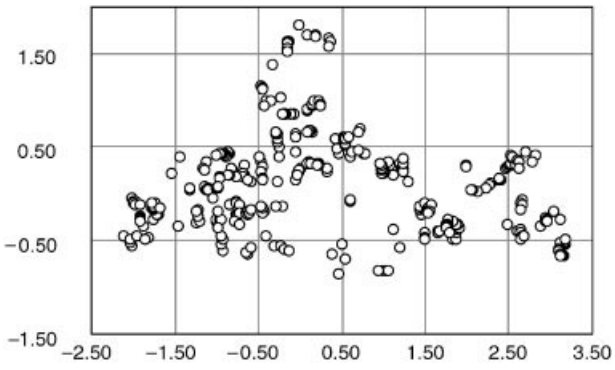

Fig. 4 Pie diagrams showing the average frequency of F5 rDNA genotypes (solid area); pooling individuals into samples follows the design described in Fig. 3. No cline was fitted for this locus because of the extreme asymmetry of the FM2 rDNA into the range of F5.

chromosome 6 coalign relatively close to the diagonal, while chromosome 1 and the mtDNA cline both deviate off the diagonal. This plot provides stronger visual confirmation of the presumed concordance between some cline pairs, and the discordance of others, but only in a qualitative manner.

To quantify these differences we performed a Bartlette's test of homogeneity for all pair-wise combinations of clines, as implemented in ANALYSE. As an 'internal control' we first tested the two recombinant forms of chromosome 2 against each other (2a and 2b in Table 3), because these are linked markers (Sites *et al*. 1995) that should be in strong linkage disequilibrium with each other. In this test they were not significantly different (Table 3). We then tested chromosome 2a with chromsome 6, with chromosome 1 and with the mtDNA cline. These last three markers were also tested against each other in all possible combinations. Table 3 shows that chromosome 2 did not differ significantly from chromosome 6, but all other combinations of clines were significantly discordant.

Because mtDNA is often discordant with nuclear loci in narrow hybrid zones (Harrison 1990), we plotted the frequency of FM2 mtDNA in five genotypic classes formed by pooling all individuals of the same class, as defined by chromosome markers, regardless of the geographical location of each individual on the sampling grid. This summary revealed that 10 of the 13 'F_1-like' lizards carried FM2 mtDNA haplotypes (Fig. 6). We tested this observed number against the expected ratio (50/50), under an assumption that all 'F_1-like' animals really are hybrids, and that mating is strictly random with respect to the chromosomal genotype of each pure parent. A small sample exact binomial test showed frequency of the FM2 haplotype to be not significantly different from the null expectation ($P \approx 0.09$), but failure to

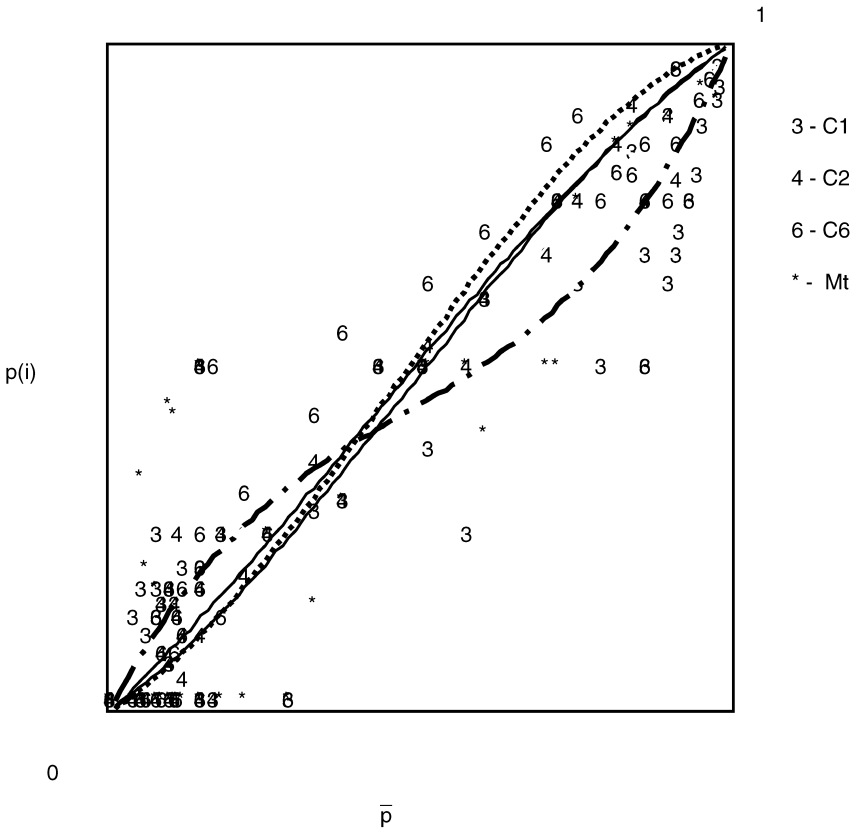

Fig. 5 Concordance of clines across the chromosomal and mitochondrial markers used in this study; p(i), the frequency of each marker is plotted against $\bar{p}$ the average frequency for all markers. Chromosomes 2 and 6 (the nearly indistinguishable solid lines) occur close to the diagonal, whereas, chromosome 1 (the dashed line) and the mtDNA (the dotted line) deviate more from the diagonal. If clines were perfectly concordant, all points would align on the diagonal. The curves show cubic polynomials of the form $p(i) = \bar{p} + 2(\bar{p})(\bar{q})[\alpha I - \beta i(p - q)]$, fitted by maximum likelihood, using all 114 sites formed by pooling over 100 m; α_l approximates a shift in position and β_l a change in width.

Clines	χ^2	d.f.	Rank	*P*-value	Bonferroni corrected *P*
Cline 2a vs. 2b	3.778	1	7	0.0518	0.0518
Cline 2a vs. 6	4.507	1	6	0.0337	0.0674
Cline 2a vs. mtDNA	28.910	1	5	< 0.00001	< 0.00001
Cline 6 vs. mtDNA	37.425	1	4	< 0.00001	< 0.00001
Cline 1 vs. 6	81.814	1	3	< 0.00001	< 0.00001
Cline 1 vs. 2	109.360	1	2	< 0.00001	< 0.00001
Cline 1 vs. mtDNA	209.873	1	1	< 0.00001	< 0.00001

Table 3 Pair-wise comparisons between all combinations of clines, using a Barlette's test for homogeneity (*P*-values are based on a χ^2 distribution)

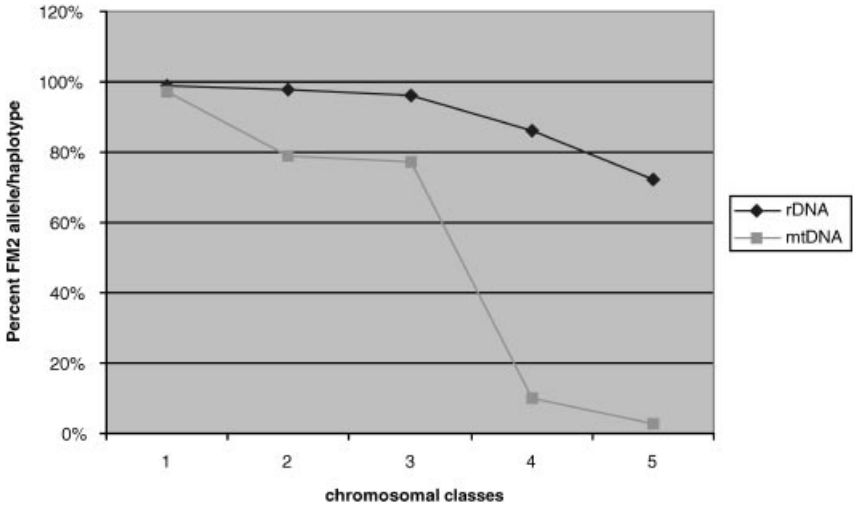

Fig. 6 Measure of introgression of the FM2 mitochondrial haplotype and the rDNA locus across five genotypic classes (as defined by the three chromosomes) of hybrid zone individuals. Class 1 = chromosomally pure FM2, class 2 = $F_1 \times$ FM2 backcrosses, class 3 = F_1-like generation, class 4 = $F_1 \times$ F5 backcrosses and class 5 = chromosomally pure F5 genotypes.

reject may be due to a small sample size of F_1-like individuals (n = 13, see Appendix) and to the weak power of the test.

Discussion

Different evolutionary scenarios may give rise to identical cline shapes in hybrid zones (Barton & Gale 1993). When multiple clines are evaluated, comparing shapes can tell us about the evolutionary processes most influencing the zone. For example, if all clines from unlinked markers are concordant in shape and position, this suggests a strong selective pressure against hybrids (Barton 1983; Barton 1986). Selection does not necessarily need to be acting against all heterozygotes at all loci because epistatic effects between loci can generate a collective selection pressure (Gavrilets 1997; Barton & Shpak 2000). Selection defined in relation to adaptation to different environments, where a geographical selection gradient determines the fitness of the genotypes, can produce strong selective pressure alone or in concert with other pressures. In all the above cases, selection will 'spill over' onto any loci in linkage disequilibrium with any selected locus. This increases the effective selection on each locus and may generate a steep step in gene frequencies that will result in strong concordance among all clines (Barton 1983; Baird 1995). Such coincident clines are known in a number of natural hybrid zones, including: cottonwood trees, *Populus* sp. (Paige *et al*. 1991); European alpine grasshoppers of the *Podisma pedestris* complex (Barton & Hewitt 1985); and the Australian grasshoppers of the *Caledia captiva* complex (Moran *et al*. 1980). Analytical and simulation studies show that cline shapes generated by endogenous and exogenous selection are indistinguishable (Kruuk *et al*. 1999). However, a very different pattern of cline shape is observed if selection is acting only weakly on loci. In this case, clines will act independently of each other rather than as a single nonrecombining unit. As such, each cline may respond to independent forces such as locus-specific selection and/or drift, and the resulting pattern may be discordance of cline shape and width, or noncoincidence of clines (Barton & Bengtsson 1986; Harrison 1990; Barton & Gale 1993; Searle 1993; Searle *et al*. 1993).

Previous studies concluded that a number of factors were influencing the genetic structure of the Tulancingo hybrid zone. Concordant cytonuclear disequilibria patterns for the mtDNA locus and the three chromosomal markers indicated that genome wide forces rather than genetic drift or locus-specific deterministic forces were

predominant in structuring the zone (Sites *et al*. 1996). Migration models suggested that continued gene flow from the parental races, combined with random mating, could account for much of the observed structure (Sites *et al*. 1996). Significant linkage disequilibria and loosely concordant clines for the three diagnostic chromosomes also hint at genome wide forces being predominant in structuring the zone (Sites *et al*. 1995). Along with continued migration of parental types into the zone, it was also found that F_1-like females suffered reduced fecundity and that there was a strong association between karyotype and habitat, so both endogenous and exogenous natural selection are important forces structuring this hybrid zone. However, not all chromosomes experienced the same selective pressures. Malassortment of chromosome 2 was found in F_1 hybrid males (Reed *et al*. 1995b) but chromosomes 1 and 6 undergo normal meiosis (Reed *et al*. 1995a).

This study provides a more in-depth look at these issues by adding the mtDNA and rDNA markers, and then statistically testing the concordance of the three chromosomal markers in all paired combinations with each other and with the mtDNA locus. Results show significant discordance between some pairs of genetic markers that was not evident in the earlier study (Sites *et al*. 1995). We conclude that different combinations of evolutionary processes, some of which had not been previously considered, contribute to the structure of this hybrid zone.

Multi-locus clines and the dominant forces shaping the structure of the Tulancingo zone

This analysis revealed that chromosomes 2 and 6 were not significantly discordant with each other in location or shape (Table 3). Sites *et al*. (1995) estimated a dispersal rate of $\sigma = 160$ m/generation for lizards in the Tulancingo area, and an average cline width of w ~830 m for the hybrid zone (across the three chromosomes combined). Under a model of endogenous selection and given the estimated dispersal rate, the maintenance of a cline with an 830-m width requires selection against the heterozygotes of $8\sigma^2/w$ (Bazykin 1969), or $\cong 29.7\%$. Under a model of exogenous selection along an environmental gradient, selection against heterozygotes would be $3\sigma^2/w$ (Haldane 1948), or $\cong 11.1\%$. Our best fits for these chromosomes reveal widths of ~800 m for each (Tables 1 and 2). Using the dispersal rates of Sites *et al*. (1995) and the single-locus cline models of Bazkin (1969) and Haldane (1948), we estimate selection against heterozygotes of 32% (endogenous) and 12% (exogenous) for each chromosome.

An interesting feature of these chromosomes, given the concordance of their clines, is the independent evidence for different forms of selection acting on each. Studies of meiotic pairing behaviour in males revealed that the simple Robertsonian heterozygotes of chromosome 6 undergo normal disjunction in F_1-like and in all classes of backcross genotypes, as do recombinant genotypes for the complex rearrangements at chromosome 2 (Reed *et al*. 1995a). However, F_1-like chromosome 2 heterozygotes (AB_d) show very high levels of trivalent/univalent configurations (48–81% of 1149 diakinetic arrays scored in eight lizards), and consequently show high frequencies of expected aneuploid gamete formation (Reed *et al*. 1995b). Similarly, there are significantly fewer ($P < 0.0006$) viable embryos in clutches of females carrying the AB_d chromosome 2 compared to parental and recombinant chromosome 2 genotypes, and no fecundity loss due to heterozygosity at chromosome 6 (Reed & Sites 1995). The influence of individual chromosomes on female fecundity is compounded by interaction effects (F_1 genotypes in general [$AB/AB_d/AB$] had significantly smaller clutch sizes, and a significantly reduced number of viable embryos, relative to all other genotypes; see Tables 2 in Reed & Sites 1995), but on balance the combined evidence suggests strong endogenous selection against chromosome 2 AB_d genotypes. In contrast, the same fitness correlates reveal no evidence of endogenous selection against the chromosome 6 AB genotypes. Tight concordance and coincidence of these markers are probably due to both an influx of parental genotypes (generating linkage disequilibium between these chromosomes), and 'spill-over' effects of strong endogenous selection from chromosome 2 onto chromosome 6 (Sites *et al*. 1995), which would maintain concordance in cline shape between them.

Chromosome 1 and the mtDNA locus were significantly discordant with clines for chromosome 2 and 6, and also with each other. Inspection of Fig. 5 also shows that the symmetries of the two differ. In confirmation of Sites *et al*. (1995), chromosome 1 metacentrics are significantly more common than average when metacentrics for other chromosomes are rare, and rarer than average when metacentrics at other chromosomes are common. In other words, the chromosome 1 cline is significantly wider and displaced in favour of F5 metacentric introgression into the FM2 race and vice-versa. Using our best-fit cline width of 1.20 km for this chromosome, and the above equations, we estimate selection against heterozygotes $\cong 14.2\%$ and 5.3%, for endogenous or exogenous components, respectively (Haldane 1948; Bazykin 1969). Reed & Sites (1995; Table 3) found evidence for significant segregation distortion in favour of AB genotypes in embryos of females of the same genotype. In two females (BYU 43088 and IBH 7518–7) heterozygous for chromosome 1, four of five and five of five embryos, respectively, were also heterozygous for chromosome 1. The earlier studies of this chromosome were not designed to assess its influence on other fitness correlates, but we conclude that locus-specific selection of some sort operates in such a manner as to cause chromosome 1 to behave independently from the chromosome 2 and 6 clines.

Finally, the mtDNA cline deviates from all others in that it is significantly wider than the overall average (Fig. 5). No evidence for nonrandom mating was revealed by earlier studies, but a nonsignificant trend was noted in the F_1-like genotype class, with 77% of the haplotypes being FM2 (Fig. 6). The sample size was quite small ($n = 13$) and more data are necessary to investigate the trend. However, it is interesting to note that F5 males are significantly larger than FM2 males of the zone; the average snout to vent length for the F5 males ($n = 22$) is 58.2 mm compared to 52.0 mm for FM2 males ($n = 17$, student's t-test, $t = 4.47$, d.f. = 36, $P < 0.0001$). If FM2 females in the hybrid zone prefer larger F5 males, F_1-like genotypes would contain more FM2 mtDNA haplotypes than expected under random mating. In at least one case, female broad-headed skinks (*Eumeces laticeps*) actively chose larger males for mating (Cooper & Vitt 1993), and such a bias in *Sceloporus grammicus* would lead to asymmetrical introgression of FM2 mtDNA haplotypes into the F5 race.

In analytical models of deterministic influences on cline shape, drift is treated as though its effects are unbiased with respect to cline shape, but it is known to widen clines (Slatkin & Maruyama 1975). Given the smaller effective population size (N_e) for nonrecombining haploid loci relative to Mendelian loci (Birky *et al*. 1983; Birky *et al*. 1989), we suggest that stochastic influences (drift) probably interact with one or more deterministic forces specific to mtDNA in dissociating this cline from others.

rDNA and possible explanations for its extensive asymmetry

The largest deviation is seen in the distribution of rDNA genotypes. The rDNA locus is located on chromosome 2; specifically, the single pair of nucleolus organizing regions (NORs) is terminal on the long arms of this pair (Reed *et al*. 1995b). This means that forces are acting strongly enough on the rDNA locus, or on a closely linked locus, to cause the rDNA cline to differ in shape and location relative to the chromosome on which it is found. This drastic deviation may be due to selection favouring the FM2 rDNA allele (asymmetric introgression), or nonreciprocal exchange (biased gene conversion) of rDNA sequences between the FM2 and F5 races. Arnold *et al*. (1987) reported extreme asymmetric distribution of Moreton rDNA in a hybrid zone between Moreton and Torresian chromosomal races of the *Caledia captiva* complex. In the *Caledia* hybrid zone, a disproportionate number of Torresian individuals, as diagnosed by chromosomal genotypes, contained Moreton rDNA.

Arnold *et al*. (1987) suggests three possible mechanisms to explain this asymmetry; two involved natural selection acting on either the entire NOR-bearing chromosomes, or only the NOR regions, and the third was biased gene conversion in favour of the Moreton rDNA. Arnold *et al*. (1988) tested these possibilities by evaluating the distribution of in-site hybridization and restriction fragment length polymorphism (RFLP) rDNA data collected from the same individuals used in the chromosome analyses. These data revealed that the rDNA RFLPs were asymmetrically distributed independent of the NOR-bearing chromosomes, indicating an asymmetric transfer of rDNA between the NORs in favour of the Moreton race.

More recently, Fuertes Aguilar *et al*. (1999) also showed biased gene conversion in the F_2 generation for nuclear rDNA in natural and artificial plant hybrids of the genus *Armeria*. Here, five of six nucleotide sites showed a tendency to homogenize polymorphic sites with a bias toward one of the parental types (*A. colourata*). A similar conversion bias operating on the *S. grammicus* rDNA genes in the Tulancingo transect, specifically in favour of the FM2 rDNA repeat, could account for the observed asymmetric distribution.

An alternative explanation for the pattern of the rDNA distribution is that there may have been a geographical shift of the zone from east to west. This shift may have moved all the other markers but left the rDNA gene frequency 'track' behind at the old locality. Martin & Cruzan (1999) showed the effect that a shifting hybrid zone can have on the movement of neutral and non-neutral markers. In a broad zone of hybridization in herbaceous perennial plants of the *Piriqueta caroliniana* complex, the within–population morphological variation was greatest toward the advancing front of introgression, and levels of genetic variation for neutral diagnostic markers were greatest in the region of initial contact and lower in areas of recent expansion. If the Tulancingo hybrid zone has shifted to the west, and if the exogenous selection was strong enough to permit all the markers together (except the rDNA) to track this shift, the expected pattern would be an eastwardly displaced FM2 rDNA cline.

When evaluating the likelihood of the three possible explanations for the observed asymmetry, we need to consider that the NOR region is located on chromosome 2 and what this implies for all scenarios. Both the 'moving zone' and 'asymmetric introgression' hypotheses require selection to act individually on either the NOR region or chromosome 2, but this will influence both because they are linked. This would not be a requirement with asymmetric gene conversion, and given the above examples and the demonstrated directionality of the rDNA gene conversion in parthenogenetic lizards of hybrid origin (Hillis *et al*. 1991), this is the most parsimonious explanation for the extreme discordance in favour of the eastern skewness of rDNA genotypes in the *S. grammicus* chromosome races.

The variety of largely independent genetic markers (mitochondrial, chromosomal, and a specific locus for a repeat family of genes) used in this study has allowed us to specify more precisely several of the different forces probably at work in shaping the structure of the Tulancingo

zone. By separating the different possible combinations of mechanisms operating on a locus-by-locus basis, we can now formulate and test more detailed hypotheses about the operation of each one.

Acknowledgements

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Appendix I

Summary of the total number of different genotypes (coded for five genetic markers as described in the text) for the 502 *Sceloporus grammicus* used in this study. Localities are those plotted in Fig. 1; HI = hybrid index (0 = pure FM2, and 8 = pure F5; based on the 3 diagnostic chromosomes), *n* = sample size for each group, mt. = mitochondrial haplotype.

		Chromosomes								Chromosomes					
Localities	HI	1	2	6	mt.	rDNA	*n*	Localities	HI	1	2	6	mt.	rDNA	*n*
Tulanc.	8	AA	AA	AA	F5	F5	15	quad 12	5	AB	AB_d	AA	FM2	FM2	1
S. Tulant.	8	AA	AA	AA	F5	F5	13		3	BB	AR_q	BB	FM2	FM2	1
	8	AA	AA	AA	F5	H	9		1	AB	B_dB_d	BB	FM2	FM2	1
	7	AB	AA	AA	F5	H	1		0	BB	B_dB_d	BB	FM2	FM2	7
quad 28	8	AA	AA	AA	F5	FM2	8	quad 13	2	AB	B_dB_d	AB	FM2	FM2	1
	8	AA	AA	AA	F5	—	2		2	BB	AB_d	BB	FM2	FM2	1
quad 24	8	AA	AA	AA	F5	H	3		1	AB	B_dB_d	BB	FM2	FM2	1
	8	AA	AA	AA	F5	FM2	8		0	BB	B_dB_d	BB	FM2	FM2	10
	8	AA	AA	AA	FM2	FM2	2		0	AB	B_dB_d	BB	F5	FM2	1
	8	AA	AA	AA	F5	—	2	quad 11	4	AB	AB_d	AB	FM2	FM2	1
	7	AB	AA	AA	F5	FM2	3		3	AB	AB_d	BB	FM2	FM2	1
	7	AB	AA	AA	F5	H	1		3	AA	B_dB_d	AB	FM2	FM2	1
quad 5	8	AA	AA	AA	F5	FM2	8		2	BB	AB_d	BB	FM2	FM2	1
	8	AA	AA	AA	F5	—	1		1	AB	B_dB_d	BB	FM2	FM2	4
	8	AA	AA	AA	F5	H	2		1	BB	B_dB_d	AB	FM2	FM2	5
	7	AB	AA	AA	F5	FM2	6		0	BB	B_dB_d	BB	FM2	FM2	18
	7	AB	AA	AA	F5	H	5	quad 14	8	AA	AA	AA	F5	FM2	3
	7	AB	AA	AA	F5	—	1		5	BB	AA	AB	F5	H	1
quad 1	8	AC	AA	AA	F5	FM2	1		3	AA	B_dB_d	AB	FM2	FM2	1
	8	AA	AA	AA	F5	FM2	2		2	AA	B_dB_d	BB	FM2	FM2	1
	8	AA	AA	AA	F5	—	2		1	AB	B_dB_d	BB	F5	H	1
	7	AB	AA	AA	F5	FM2	1		1	AB	B_dB_d	BB	F5	FM2	1
quad 2	8	AA	AA	AA	F5	FM2	9		1	AB	B_dB_d	BB	FM2	FM2	1
	8	AA	AA	AA	F5	—	1		1	BB	B_dB_d	AB	FM2	FM2	1
	7	AB	AA	AA	F5	FM2	3		0	BB	B_dB_d	BB	FM2	FM2	9
quad 3	8	AA	AA	AA	F5	FM2	4		0	BB	B_dB_d	BB	FM2	H	3
	8	AA	AA	AA	F5	—	1		0	BB	B_dB_d	BB	F5	FM2	1
quad 4	8	AA	AA	AA	F5	FM2	5	quad 18	4	BB	AA	BB	FM2	FM2	2
	8	AA	AA	AA	F5	H	3		2	BB	R_qR_q	BB	FM2	FM2	1
quad 21	8	AA	AA	AA	F5	FM2	12		1	AB	B_dB_d	BB	FM2	FM2	5
	8	AA	AA	AA	F5	H	15		1	AB	B_dB_d	BB	FM2	—	1
	8	AA	AA	AA	F5	F5	1		0	BB	B_dB_d	BB	FM2	FM2	7
	7	AB	AA	AA	F5	H	5	quad 17	8	AA	AA	AA	F5	—	1
	7	AB	AA	AA	F5	FM2	1		4	AB	AB_d	AB	FM2	FM2	1
	0	BB	B_dB_d	BB	FM2	FM2	1		3	BB	AB_d	AB	FM2	FM2	1
quad 20	8	AA	AA	AA	F5	—	2		2	AB	B_dB_d	AB	FM2	FM2	1
	7	AB	AA	AA	F5	F5	1		1	AB	B_dB_d	BB	FM2	FM2	1
	7	AB	AA	AA	FM2	H	1		1	AB	B_dB_d	BB	FM2	FM2	1
	4	AB	AR_c	BB	FM2	FM2	3		1	BB	B_dB_d	AB	FM2	FM2	1
quad 19	4	AB	AB_d	AB	FM2	FM2	1		0	BB	B_dB_d	BB	FM2	FM2	11
	0	BB	B_dB_d	BB	FM2	FM2	3		0	BB	B_dB_d	BB	FM2	—	1
	0	BB	B_dB_d	BB	FM2	—	1	quad 16	8	AA	AA	AA	F5	FM2	1
Point C	0	BB	B_dB_d	BB	FM2	FM2	4		7	AB	AA	AA	F5	FM2	1
quad 6	8	AA	AA	AA	F5	FM2	13		6	BB	B_dR_q	AB	FM2	FM2	1
	8	AA	AA	AA	FM2	FM2	1		6	AB	AR_q	AA	FM2	FM2	1
	7	AB	AA	AA	F5	FM2	3		5	AB	AR_c	AB	FM2	FM2	1
	7	AA	AA	AB	F5	FM2	1		4	AB	AB_d	AB	FM2	FM2	1
	7	AB	AA	AA	F5	F5	1		1	BB	B_dB_d	AB	FM2	FM2	1
quad 7	8	AA	AA	AA	F5	H	1		1	AB	B_dB_d	BB	FM2	FM2	1
	3	BB	AR_c	BB	FM2	—	1		0	BB	B_dB_d	BB	FM2	FM2	3
	2	BB	B_dB_d	AA	FM2	FM2	1								

Appendix I *Continued*

		Chromosomes								Chromosomes					
Localities	HI	1	2	6	mt.	rDNA	*n*	Localities	HI	1	2	6	mt.	rDNA	*n*
quad 22	8	AA	AA	AA	F5	—	1	quad 15	8	AA	AA	AA	F5	FM2	8
	8	AA	AA	AA	F5	FM2	5		8	AA	AA	AA	FM2	FM2	1
	8	AA	AA	AA	F5	H	1		8	AA	AA	AA	F5	–	1
	8	AA	AA	AA	FM2	FM2	1		7	AB	AA	AA	F5	FM2	1
	7	AB	AA	AA	FM2	F5	2		7	AA	AA	AB	F5	FM2	1
	7	AA	AA	AB	F5	FM2	2		6	AA	AA	BB	FM2	FM2	1
	6	AB	AR_c	AA	F5	FM2	1		6	AB	AA	AB	F5	FM2	1
	6	AB	AA	AB	F5	FM2	1		6	AB	AA	AB	FM2	H	1
	5	AB	AR_q	AB	F5	—	1		5	AB	AR_c	AB	FM2	H	1
	4	AB	AB_d	AB	FM2	FM2	3		5	AB	AB_d	AA	F5	FM2	1
	4	AB	AB_d	AB	F5	FM2	1		4	AB	AB_d	AB	F5	H	1
	2	BB	AB_d	BB	F5	—	1		2	BB	B_dR_c	AB	FM2	FM2	1
	1	AB	B_dB_d	BB	F5	FM2	1		2	AB	B_dB_d	AB	F5	FM2	1
	1	BB	B_dB_d	AB	F5	FM2	1		1	BB	B_dR_c	BB	F5	FM2	1
quad 8	8	AA	AA	AA	F5	FM2	1		0	BB	B_dB_d	BB	FM2	FM2	2
	7	AA	AA	AB	F5	FM2	1	quad 23	8	AA	AA	AA	F5	FM2	5
	7	AB	AA	AA	F5	FM2	3		7	AA	AR_c	AA	F5	FM2	3
	7	AB	AA	AA	F5	—	1		7	AB	AA	AA	F5	FM2	2
	6	AB	AR_c	AA	F5	FM2	3		7	AA	AA	AB	F5	FM2	7
	5	BB	AR_c	AA	F5	FM2	1		6	AA	AR_c	AB	F5	FM2	1
	5	AB	AB_d	AA	FM2	FM2	1		6	AA	AB_d	AA	F5	FM2	1
	5	AB	AR_q	AB	FM2	FM2	1	point B	8	AA	AA	AA	F5	FM2	9
	4	AB	AB_d	AB	F5	FM2	1		7	AA	AA	AB	F5	FM2	1
	4	AB	AB_d	AB	FM2	FM2	3	quad 25	0	BB	B_dB_d	BB	FM2	FM2	13
	3	BB	AB_d	AB	FM2	FM2	1	quad 26	0	BB	B_dB_d	BB	FM2	FM2	12
	2	AB	B_dB_d	AB	FM2	FM2	1	quad 27	0	BB	B_dB_d	BB	FM2	FM2	11
	1	BB	B_dB_d	AB	FM2	FM2	2	M.Rodr.	0	BB	B_dB_d	BB	FM2	FM2	8
	1	BB	B_dB_d	AB	FM2	H	1	quad 10	3	AA	B_dB_d	BB	FM2	—	1
	1	AB	B_dB_d	BB	FM2	FM2	1		2	BB	AB_d	BB	FM2	FM2	3
	0	BB	B_dB_d	BB	FM2	FM2	4		1	AB	B_dB_d	BB	FM2	FM2	2
quad 9	5	BB	AA	AB	FM2	FM2	1		0	BB	B_dB_d	BB	FM2	FM2	9
	2	BB	B_dB_d	AA	FM2	FM2	1		0	BB	B_dB_d	BB	F5	—	1
	2	AB	B_dB_d	AB	FM2	FM2	2		0	BB	B_dB_d	BB	FM2	—	2
	1	BB	B_dB_d	AB	F5	FM2	1		0	BB	BB_d	BB	F5	FM2	3
	1	AB	B_dB_d	BB	FM2	FM2	1								
	1	AB	B_dB_d	BB	F5	FM2	1								
	0	BB	B_dB_d	BB	FM2	FM2	10								