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Parapatric Hybridization between Chromosome Races of the *Sceloporus grammicus* Complex (Phrynosomatidae): Structure of the Ajusco Transect

ELISABETH ARÉVALO, GUSTAVO CASAS, SCOTT K. DAVIS, GUILLERMO LARA, AND JACK W. SITES, JR.

Genetic and morphological variation in the *Sceloporus grammicus* complex was examined along an elevational transect in the Sierra del Ajusco, southwest of Mexico City. A total of 122 lizards was collected from six localities beginning at 2400 m and set at every 200 m of elevation; and a subset of 107 animals were examined for their chromosomal number, 10 morphological characters and three protein systems. A subset of this sample was also examined for ribosomal and mitochondrial DNA restriction sites. Three chromosomal races were found, with 2n = 32 (LS) characteristic of low elevation, 2n = 34 (F6) characteristic of intermediate elevation, and 2n = 32 (HS) characteristic of high elevation. A parapatric contact zone was found between locality four (F6) and five (HS). A hybrid index score obtained with the diagnostic markers of these races showed a total of 42 pure F6, 43 pure HS, six possible backcrosses to F6, and two F_1 individuals. Different levels of introgression were found for the different marker systems. Two different classes of markers, allozyme and mtDNA, showed asymmetrical gene flow from the F6 into the LS. The asymmetrical flow of the F6 mtDNA into the LS distribution was very pronounced with the F6 haplotypes occurring at a frequency of 0.5 at the LS locality. The rDNA markers showed symmetrical flow to both sides of the F6 and HS races. Taxonomic implications of the levels of morphological and molecular divergence are discussed, and the origins of the F6-HS and F6-LS contact zones are interpreted to represent a secondary contact.

Se examinó variación genética y morfológica en el complejo *Sceloporus grammicus* a lo largo de un transecto elevacional en la Sierra del Ajusco, al suroeste de la ciudad de México. Un total de 122 lagartijas se colectaron en seis localidades empezando a los 2400 m y establecidas a cada 200 m de altitud, y una submuestra de 107 animales fue examinada en cuanto a su número cromosómico, diez caracteres morfológicos y tres sistemas de proteínas. Una selección de esta muestra también fue examinada para sitios de restricción de ADN mitocondrial y ribosomal. Tres razas cromosómicas fueron encontradas, con 2n = 32 (LS) característico de bajas altitudes, 2n = 34 (F6) característico de altitudes intermedias y 2n = 32 (HS) característico de mayores altitudes. Una zona de contacto parapátrico se encontró entre la localidad cuatro (F6) y cinco (HS). Se obtuvo un índice de híbridos con los tres marcadores diagnósticos, el cual demostró un total de 42 F6 puros, 43 HS puros, seis posibles retrocruzas a F6 y dos individuos F_1. Diferentes niveles de introgresión fueron encontrados para los diferentes sistemas marcadores. Dos clases de marcadores, alozimas y ADN mitocondrial, mostraron un flujo asimétrico de F6 hacia LS. El flujo asimétrico en el ADN mitocondrial de F6 hacia la distribución de LS fue muy pronunciada, con los haplotipos de F6 presentes con una frecuencia de 0.5 en la localidad de LS. Por otro lado, los datos del ADN ribosomal mostraron un flujo simétrico a ambos lados de las razas F6 y HS. Implicaciones taxonómicas de los niveles de divergencia morfológica y molecular son discutidos, y el origen de los contactos entre F6-HS y F6-LS se interpretan como un contacto secundario.

THE existence of parapatric contacts between closely related species has attracted the attention of many evolutionary biologists in recent years, primarily for two reasons. First, such zones of hybridization are frequently viewed as "windows" on evolutionary processes; and second, the maintenance of abrupt discontinuities in characters in spite of ongoing hy-

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bridization makes the populations involved difficult to classify under conventional taxonomic schemes (Harrison, 1991). It is precisely these kinds of zones, i.e., those occurring as abrupt discontinuities between otherwise relatively homogenous entities, that imply conflict between gene flow and selection toward alternative stable equilibria and may, therefore, provide information on both processes (Barton and Hewitt, 1989). There is vast literature documenting cases of hybrid zones in both plants and animals and an expanding literature on hybrid zone theory (see reviews by Barton and Hewitt, 1985, 1989; Harrison, 1991); but the origin and fate of such zones, including their role in speciation processes, continue to be vigorously debated (Otte and Endler, 1989).

Several factors contribute to the nature of these debates, though many stem from difficulties deriving from comparing an extremely heterogenous pool of studies. Many early studies, for example, were undertaken prior to the development of any theoretical framework and were frequently based on single classes of characters (mostly morphological, and/or color differences between hybridizing taxa; see tables in Barton and Hewitt, 1985).

In addition, the application of new biochemical and molecular approaches has revealed patterns that were not apparent in earlier morphological studies, thereby providing important insights into the genetics of hybrid-zone interactions. Finally, the geographic dimensions of hybrid zones and differences in the organisms involved (in density, dispersal, etc.) frequently place constraints on sampling protocols, which in turn may limit statistical inferences if sample sizes are small (frequently the case in very narrow zones; see data in Barton and Hewitt, 1985). Nevertheless, important insights can be gained from even modest sample sizes when multiple data sets are collected from single transects (recent examples in Baker et al., 1989; Shaw et al., 1990; Dowling and Hoeh, 1991). Where conditions permit, sampling of different transects or points of contact at different geographic locations within the same species complex will provide comparative perspectives regarding the structure and consequences of hybrid zones (see examples by Szymura and Barton, 1986, 1991; Wake et al., 1989). This paper is the first of a pair of reports on two transects between several combinations of chromosome races of the *Sceloporus grammicus* complex in central Mexico.

Recent population cytogenetic studies of this complex (Porter and Sites, 1986; Arévalo et al., 1991) have revealed the presence of eight distinct chromosome races (= cytotypes) of *S. grammicus* in central Mexico and several points of parapatric contact between different combinations of populations. Figure 1 updates the distribution map of Arévalo et al. (1991) and shows locations of these seven contacts (identified by upper case letters A–G). The chromosome races plotted in Figure 1 include Low Standard [LS, 2n = 32; all diploid numbers given in this and the companion paper (Sites et al., 1993) are those of females; males have one fewer microchromosome due to a sex chromosome heteromorphism], and High Standard (HS, 2n = 32), which are identical karyotypically but divergent at other levels of genome organization (Sites and Davis, 1989; Sites et al., 1988), Fission 6 (F6, 2n = 34), Fission 5 (F5, 2n = 34), Fission 5 + 6 (F5 + 6, 2n = 36), and three "Multiple Fission" races (FM3, 2n = 38; FM1, 2n = 40–42; and FM2, 2n = 44–46; see details in Arévalo et al., 1991). Two of the hybrid zones have previously been studied in some detail; the 32 × 46 (LS × FM2) zone identified by letter C in Figure 1 was described from several chromosomal markers by Hall (1973), and the 32 × 34 (HS × F6) zone east of Mexico City (letter D in Fig. 1) was described in some detail by Hall and Selander (1973) on the basis of both chromosome and allozyme markers. We have established transects through zones B and F, which collectively provide two comparative perspectives on hybridization and patterns of gene flow within the *S. grammicus* complex. Zone F involves the same two races examined by Hall and Selander (1973; 32 × 34), but at a different geographic location on the opposite side of the Valley of Mexico (see D and F in Fig. 1). Zone B (F5 × FM2) involves hybridization between populations showing substantially greater levels of chromosomal divergence, a minimum of five fixed differences (34 × 46), relative to a single fixed rearrangement in the 32 × 34 contact. Because the role of chromosomal rearrangements in speciation is still unsettled (Sites and Moritz, 1987), and because Hall and Selander (1973) suggested that reduced fitness in backcross combinations in the 32 × 34 zone was sufficient to restrict genetic introgression, comparison of patterns of gene flow between these zones and the 34 × 46 contact, should provide insight into the relative contribution of chromosomal divergence to restriction of gene flow. One general prediction that can be made about the genetic structures of these zones is that, if selection against introgression is spread across many loci on the macrochromosomes (which comprise most of the genome), then both zones could be barriers to gene flow (Barton, 1983). Analytical studies such as those of Walsh (1982),

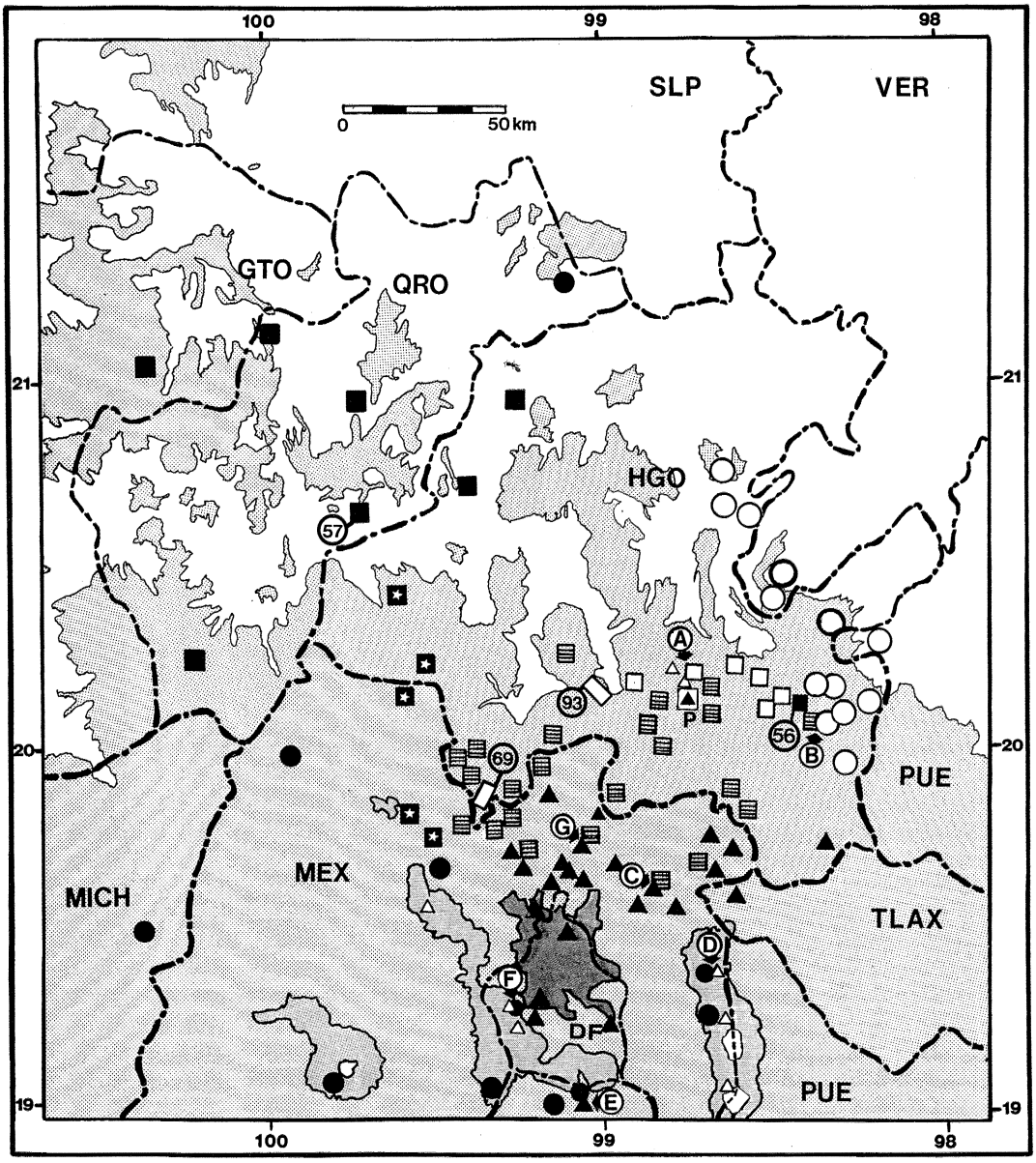


Fig. 1. Distribution map of eight chromosome races of *Sceloporus grammicus* in central Mexico. Criteria for distinguishing each race (=cytotype) are given in Arevalo et al. (1991), but this map includes new localities for the F5 and F5 + 6 cytotypes. Cytotypes are denoted by the following symbols: closed triangles = LS, open triangles = HS; closed circles = F6; open circles = F5; closed squares = F5 + 6; open squares = FM3; squares with stars = FM1; and squares with cross-hatching = FM2 (see text for details). Highly chromosomally polymorphic populations are identified by numbers (56, 57, 69, and 93) that are described in Arévalo et al. (1991). Capital letters in circles show all hybrid zones identified on the basis of chromosome markers: A, HS × FM3; B, F5 × FM2; C, LS × FM2 (same for G); D, F6 × HS (same for F); and E, LS × F6. Dashed lines and abbreviations denote the following political units: DF = Distrito Federal; GTO = Guanajuato; HGO = Hidalgo; MEX = Mexico; MICH = Michoacan; PUE = Puebla; QRO = Queretaro; TLAX = Tlaxcala; VER = Veracruz; P = city of Pachuca (in the state of Hidalgo); and darkest shading identifies Mexico City. The 2000, 3000, and 4000 m contours are identified by extensive gray shading, limited fine-grained gray, and white volcanic peaks.

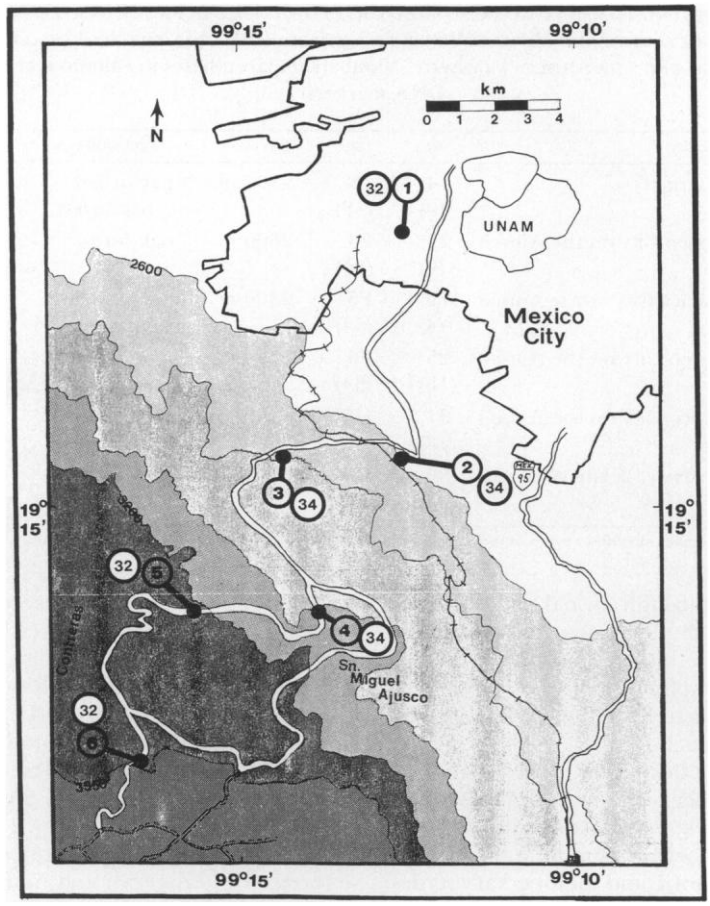


Fig. 2. Detailed topography of Ajusco transect (hybrid zone F in Fig. 1) along Ajusco-Santiago Tianguistengo Hwy, beginning with the first locality at a low elevation site, and proceeding up the north-facing Slope of the Sierra del Ajusco. Numbers connected to solid circles are localities defined in Table 1, and second series of numbers identifies chromosome race at each locality. Shaded areas identify successively higher 200 m contours, from lightest to darkest tones, and UNAM identifies the campus of the Universidad Nacional Autónoma de México.

which assume a small degree of underdominance for each rearrangement, predict that the accumulation of multiple rearrangements in the same place can form a strong barrier to gene flow. If reduced fitness in hybrid and/or backcross genotypes is due to the causes suggested by Walsh (1982) then, the 34 × 46 contact should be a more effective barrier to gene flow than the 32 × 34 contact. The objectives of these studies are to (1) compare overall patterns of gene flow at two different locations (D and F in Fig. 1) in the 32 × 34 (HS × F6) contacts, on the basis of identical chromosomal and allozyme markers; and (2) compare one of the 32 × 34 hybrid zones (locality F in Fig. 1) to the 34 × 46 zone (locality B) for extent of divergence in morphological versus molecular markers, and the extent and symmetry of introgression across both zones for single-copy and multicopy nuclear markers, and matrilineal mitochondrial markers (Sites et al., 1993).

Materials and Methods

Field sampling.—During the summer of 1986, 122 lizards were collected along an altitudinal transect on the north side of the Sierra del Ajusco, along the Ajusco's Panoramic highway, just southwest of Mexico City (zone F in Fig. 1). Six localities were sampled approximately every 200 m, starting at 2400 m in elevation, and ascending the Ajusco highway to an elevation of 3350 m, on the Ajusco-Santiago Tianguistengo Hwy (2 km W of the Panoramic highway). This transect (now referred to as the Ajusco transect) begins in a perturbed oak forest at the lower

TABLE 1. LOCALITIES (NUMBERED TO CORRESPOND TO SITES IDENTIFIED IN FIG. 2), SAMPLE SIZES (n), CYTOTYPE (2n), ELEVATION, VEGETATION AND MUSEUM VOUCHER NUMBERS (IBH) FOR THE SIX LOCALITIES OF *Sceloporus grammicus* SAMPLED ALONG THE AJUSCO TRANSECT. Numbers in parentheses in column n are sample sizes for DNA markers.

Locality	n	2n	Elevation	Vegetation type	Voucher numbers*
1. Pedregal de San Angel	14 (9)	LS (32)	2350 m	perturbed oak forest	6843, 6849
2. Km 6.5 from Mexico City on the Ajusco Panoramic Hwy	23 (9)	F6 (34)	2600 m	oak forest	6836, 6839, 6842, 6846
3. Km 10.5 from Mexico City on the Ajusco Panoramic Hwy	9 (3)	F6 (34)	2800 m	perturbed pine forest	6837, 6840, 6847
4. Km 18 from Mexico City on the Ajusco Panoramic Hwy	25 (10)	F6 (34)	3000 m	pine forest	6838
5. Km 24 from Mexico City on the Ajusco Panoramic Hwy	31 (18)	HS (32)	3200 m	pine forest	6841, 6845
6. S. Tianguistengo Hwy, 2 km W of the Ajusco Panoramic Hwy	20 (18)	HS (32)	3350 m	fir forest	6844 6848

* A single IBH catalog number identifies a series of individuals when all were collected at the same locality and date.

localities, passes through an oak-pine community at intermediate elevations, and terminates in fir forest at the higher localities. Figure 2 shows the topographic detail of this transect, and Table 1 summarizes sample sizes and ecological details of each locality.

Lizards were collected at each locality by stunning with rubber bands or by grabbing them by hand under bark. For most lizards captured, testes and bone marrow from long bones were used to obtain mitotic and meiotic karyotypes. Skeletal muscle, liver, kidney, heart, and lung were stored in liquid nitrogen for further allozyme and molecular analysis, although juveniles often did not yield enough tissue for all analyses. Chromosomal and morphological studies were performed at the Universidad Nacional Autónoma de México (UNAM) in Mexico City, the allozyme study at Brigham Young University (BYU), and the rDNA and mtDNA surveys at Texas A&M University (TAMU). Several morphological counts and measurements were taken from the voucher specimens, always by the same investigator (G. Lara), for morphological analyses. All specimens were then deposited as museum vouchers in the research collection of the Instituto de Biología, Herpetología (IBH), at Universidad Nacional Autónoma de México in Mexico City (see Table 1). The CAP prefix found in Table 2 refers to individual field catalog numbers of C. A. Porter, as some samples were catalogued in lots identified by a single IBH number.

Morphological data.—A total of 20 meristic characters were recorded from all individuals collected along the Ajusco transect, including the number of Auricular Lobules (AL), Dorsal Spots (DS, taken from posterior edge of parietal to a point immediately between hind legs), Frontoparietals (FP), Infralabials (IL), Internal Labiomentals (ILM), Labiomentals (LM), Lorilabials (LL), Subdigital Lamellae (SDL, counted on fourth toe of hind foot), Supralabials (SL), and Supraoculars (SO) (see Fig. 2 in Smith, 1939; for identification of head scales). These characters are all bilateral, and in all cases, counts were taken from both sides of each animal and then averaged to provide a single value per character per animal.

These 10 variables were analyzed with two Statistical Analysis Systems software routines (SAS Inst., 1988). First, a stepwise discriminant analysis (SDA) was used to identify and weight those variables that would maximize separation of samples between localities at a 0.15% significance level. The SDA identified seven different characters (AL, DS, FP, IL, LL, SL, and SO) that were used in a canonical discriminant analysis (CDA). The canonical discriminant functions provided by the CDA are linear combinations of the original variables which summarize between-group differences along orthogonal axes, such that the first function explains the greatest proportion of the between-group variation, the second function explains the second largest component of this variation, and so on. This analysis provided a graphical expression of the morphological relationships between the three cytotypes at the different localities, but all individuals were classified a priori and grouped on the basis of genotype, regardless of locality.

Chromosomal data.—Mitotic and meiotic karyotypes were obtained from bone marrow and testes (adult males), respectively, for a subset of 107 specimens collected along the six localities of the transect following protocols of Porter and Sites (1985). At least three cells were scanned from each individual and photographed on a Zeiss Photomicroscope II at 400×. Chromosomal races are defined for each locality in Table 1.

Protein electrophoretic data.—The allozyme survey of Sites et al. (1988) identified protein loci showing fixed differences or polymorphisms between the cytotypes involved in hybridization along the Ajusco transect. The enzyme systems screened were aspartate aminotransferase (AAT, E.C. 2.6.1.1), glycerol-3-phosphate dehydrogenase (G3PDH, E.C. 1.2.1.8), L-lactate dehydrogenase (LDH, E.C. 1.1.1.27), and superoxide dismutase (SOD, E.C. 1.15.1.1; enzyme abbreviations and E.C. numbers are those summarized in Buth and Murphy, 1990). AAT, LDH, and SOD are multilocus enzyme systems, but only two loci, sAat-A and Ldh-B, were diagnostic for these cytotypes i.e., fixed for alternative alleles between races (for details, see Sites, et al., 1988). These two markers were resolved on the Tris-borate-EDTA II buffer of Buth and Murphy (1990).

The extent of gene flow was quantified by direct count of introgressed allozymes/chromosomes for the three diagnostic markers (sAat-A, Ldh-B, and chromosome pair 6). This provided for a direct comparison with the Rio Frio zone described by Hall and Selander (1973). The BIOSYS-I software package of Swofford and Selander (1981) was used to estimate allozyme-based Nei (1978) genetic distances for the allozyme data, and the distance matrix was converted to a UPGMA phenogram to group sample localities by overall levels of similarity.

DNA restriction site data.—Genomic DNA was isolated and extracted from a small fragment of skeletal muscle, following the procedure described by Hillis and Davis (1986). Samples from 67 individuals were subject to digestion by seven hexanucleotide restriction enzymes (Bam HI, Bgl II, Bst EII, Dra I, Kpn I, Nco I, and Pvu II) for detection of restriction fragment length polymorphisms (RFLP); and the RFLP patterns were used to infer site differences between individuals, using the restriction site maps of Sites and Davis (1989). Digestions were performed in a 37 C water bath (except Bst E II, which was incubated at 55 C), for six hours in 50 ml reactions. Following digestion by restriction enzymes, genomic DNA was separated by electrophoresis on 0.8% agarose gels, with a lambda-phage molecular weight standard. Gels were then blotted on nitrocellulose filters, and the filters were sequentially hybridized with the rDNA and mtDNA probes, pGB28S, pGB18S and λSgmt4, respectively, as described by Sites and Davis (1989). Both mtDNA and rDNA genotypes were designated by capital letters for RFLP patterns generated by each diagnostic restriction enzyme in the order given above (alphabetical). Different mtDNA haplotypes and rDNA repeat types were found for each race and were defined by a set of seven alphabetical characters (for the seven enzymes) that summarized restriction site differences among individuals. The first type was arbitrarily assigned seven "A" characters (AAAAAAA), and alternative fragment patterns were designated by altering letters for the appropriate enzyme. For example, a genotype designated AAAAAAB is one differing from the first type by having an alternative RFLP for the enzyme Pvu II; one designated AABAAAC differs in having an alternative RFLP for the enzyme Bst EII, and a third pattern for PvuII; and so on. In this manner, the mtDNA and rDNA genotypes were summarized by locality across the Ajusco transect.

Statistical analyses of the mtDNA restriction site data were carried out with version 4.0 of the REAP program (Restriction Enzyme Analysis Package, McElroy et al., 1991). Subroutine D was used to estimate nucleotide divergence (d_r as per Nei and Tajima, 1981; Nei and Miller, 1990, equation 4) for all pairwise combinations of mtDNA haplotypes, and subroutine DSE estimated the standard errors associated with all pairwise d_r values (Nei and Tajima, 1983; Nei, 1987, equations 5.41, 5.44, and 5.51). MtDNA haplotypes were then grouped by locality, and a d_r distance matrix was produced for all pairwise combinations of localities. The d_r values in this matrix were weighted by within-locality haplotype frequencies, and clustered using UPGMA. To test for between-locality heterogeneity of mtDNA haplotypes frequencies, Monte Carlo simulations were used to evaluate the extent of heterogeneity (assessed through χ^2 analysis) in the original data matrix compared to that estimated from repeated randomizations of the original matrix (see Roff and Bentzen, 1989). Repeated randomizations of the original matrix allow generation of a mean χ^2 based on chance alone, and then the probability of encountering a χ^2 value as large as that calculated for the original matrix can be determined. This approach minimizes the effects of large num-

TABLE 2. VARIATION IN KARYOTYPIC, PROTEIN, AND MOLECULAR (mtDNA, rDNA) MARKERS IN THE AJUSCO TRANSECT POPULATIONS OF *Sceloporus grammicus*. Sample (Loc) numbers refer to localities in Table 1 and Figure 2; HI = hybrid index score (based on three diagnostic scn markers identified in bold type, specific isozyme loci symbolized by Ldh and Aat are Ldh-B and sAat, respectively; * = F_1 hybrids); and ID = CAP catalog numbers of individual animals. Restriction enzymes used as molecular markers denote letter code of each genotype from left to right in the following order: 1 = Bam HI; 2 = Bgl I; 3 = Bst EII; 4 = Pvu II; 5 = Dra I (rDNA only); 6 = Nco I; 7 = Kpn I. Columns "mtDNA type" and "rDNA type" identify general categories of genotypes (LS, F6, etc.), whereas columns "mtDNA class" and "rDNA class" identify all specific genotypes.

			sc nuclear markers										
Loc	HI	ID	P6	Ldh	Aat	Sod1	Sod2	mtDNA type	mtDNA haplotype	mtDNA class	rDNA genotype	rDNA type	rDNA class
1	—	1465	**AA**	**BB**	**BB**	AA	AA	—	—	—	—	—	—
	—	1466	**AA**	**BB**	**AA**	AA	AA	—	—	—	—	—	—
	—	1467	**AA**	**BB**	**AA**	AA	AA	—	—	—	—	—	—
	—	1468	**AA**	**BB**	**AB**	AA	AA	—	—	—	—	—	—
	—	1469	**AA**	**BB**	**AB**	AA	AA	—	—	—	—	—	—
	—	1470	**AA**	**BB**	**AA**	AA	AA	LS	**BAADBA**	I	AAAAAAA	LS	I
	—	1471	**AA**	**BB**	**AA**	AA	AA	F6	**AABAAA**	III	BCBBBBB	LSXF6	II
	—	1472	**AA**	**BB**	**AA**	AA	AA	LS	**BBADCA**	II	AAAAAAA	LS	I
	—	2158	**AA**	**BB**	**AA**	AA	AA	F6	**AABAAA**	III	AAAAAAA	LS	I
	—	2159	**AB**	**BB**	**BB**	AA	AA	F6	**AABAAA**	III	AAAAAAA	LS	I
	—	2160	**AA**	**BB**	**AB**	AA	AA	LS	**BBADCA**	II	AAAAAAA	LS	I
	—	2161	**AA**	**BB**	**AA**	AA	AA	F6	**AABAAA**	III	AAAAAAA	LS	I
	—	2162	**AA**	**BB**	**AA**	AA	AA	LS	**BBADCA**	II	AAAAAAA	LS	I
	—	2163	**AA**	**BB**	**AA**	AA	AA	F6	**AABAAA**	III	AAAAAAA	LS	I
2	**0**	1406	**BB**	**BB**	**BB**	BB	BB	—	—	—	—	—	—
	0	1407	**BB**	**BB**	**BB**	AA	AA	F6	**CABAAA**	IV	AEAAAAB	F6	III
	0	1408	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	1409	**BB**	**BB**	**BB**	AA	AA	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	1410	**BB**	**BB**	**BB**	AA	AA	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	1411	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	1	1412	**BB**	**BB**	**AB**	AB	AB	—	—	—	—	—	—
	2	1413	**BB**	**BB**	**AA**	AA	AA	—	—	—	—	—	—
	0	1414	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1415	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1416	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1448	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	1464	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	1	2140	**BB**	**BB**	**AB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	2141	**BB**	**BB**	**BB**	AA	AA	F6	**AABAAA**	III	AEAAAAB	F6	III

TABLE 2. CONTINUED.

Loc	HI	ID	sc nuclear markers					mtDNA type	mtDNA haplotype	mtDNA class	rDNA genotype	rDNA type	rDNA class
			P6	Ldh	Aat	Sod1	Sod2						
	0	2142	**BB**	**BB**	**BB**	AA	AA	—	—	—	—	—	—
	1	2143	**BB**	**BB**	**AB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
3	**0**	1418	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1419	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1420	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1421	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	1449	**BB**	**BB**	**BB**	AA	AA	—	—	—	—	—	—
	0	1450	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	2145	**BB**	**BB**	**BB**	AB	AB	F6	**CABAAA**	IV	ACACAAB	F6	IV
4	**0**	1423	**BB**	**BB**	**BB**	AA	AA	—	—	—	—	—	—
	0	1424	**BB**	**BB**	**BB**	—	—	—	—	—	—	—	—
	0	1425	**BB**	**BB**	**BB**	AA	AA	F6	**CABAAA**	IV	AEACAAB	F6	V
	0	1426	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	1427	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1428	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1429	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	1	1430	**BB**	**AB**	**BB**	AB	BB	F6	**CABADA**	V	AEAAAAB	F6	III
	0	1431	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	1432	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	CEAAAAB	F6	VI
	0	1433	**BB**	**BB**	**BB**	AB	BB	—	—	—	—	—	—
	0	1434	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1435	**BB**	**BB**	**BB**	AB	BB	—	—	—	—	—	—
	0	1436	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1437	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1438	**BB**	**BB**	**BB**	AB	BB	F6	**AABAAA**	III	DBBCBBB	F6XHS	VII
	0	1439	**BB**	**BB**	**BB**	AB	BB	—	—	—	—	—	—
	0	1440	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	1	1441	**BB**	**AB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1442	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1443	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1444	**BB**	**BB**	**BB**	AB	AB	F6	**AABAAA**	III	AEAAAAB	F6	III
	0	1445	**BB**	**BB**	**BB**	AB	AB	—	—	—	—	—	—
	0	1446	**BB**	**BB**	**BB**	AB	AA	F6	**AABAAA**	III	AEAAAAB	F6	III

TABLE 2. CONTINUED.

Loc	HI	ID	sc nuclear markers					mtDNA type	mtDNA haplotype	mtDNA class	rDNA genotype	rDNA type	rDNA class
			P6	Ldh	Aat	Sod1	Sod2						
5	6	1451	AA	AA	AA	AB	AA	—	—	—	—	—	—
	6	1452	AA	AA	AA	AB	AA	HS	ACAADA	VI	DDBBBBB	HS	VIII
	6	1453	AA	AA	AA	AB	AA	—	—	—	—	—	—
	6	1454	AA	AA	AA	AB	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	1455	AA	AA	AA	AB	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	1456	AA	AA	AA	AB	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	1457	AA	AA	AA	AB	AB	—	—	—	—	—	—
	6	1458	AA	AA	AA	AA	AA	—	—	—	—	—	—
	6	1460	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBCBBB	HS	X
	3*	1461	AB	AB	AB	AA	AA	—	—	—	—	—	—
	6	1462	AA	AA	AA	AA	AA	—	—	—	—	—	—
	3*	1463	AB	AB	AB	AA	AA	—	—	—	—	—	—
	6	2123	AA	AA	AA	AA	AA	HS	ACAADA	VI	DDBBBBB	HS	VIII
	6	2124	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBCBBB	HS	X
	6	2125	AA	AA	AA	AB	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2126	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2127	AA	AA	AA	AB	AA	—	—	—	—	—	—
	6	2128	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2129	AA	AA	AA	AA	AA	HS	ACABDA	VII	DBBBBBB	HS	IX
	6	2130	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2131	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2132	AA	AA	AA	AA	AA	HS	ACABDA	VII	DBBBBBB	HS	IX
	6	2133	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2134	AA	AA	AA	AA	AA	HS	ACABDA	VII	DBBBBBB	HS	IX
	6	2135	AA	AA	AA	AA	AA	HS	ACABDA	VII	DBBBBBB	HS	IX
	6	2136	AA	AA	AA	AA	AA	HS	ACAADA	VI	DDBBBBB	HS	VIII
6	6	2112	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2113	AA	AA	AA	AA	AA	—	—	—	—	—	—
	6	2114	AA	AA	AA	AA	AA	HS	ACABDA	VII	DBBBBCB	HS	XI
	6	2115	AA	AA	AA	AA	AA	HS	ACACDA	VIII	DBBBBBB	HS	IX
	6	2116	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2117	AA	AA	AA	AB	AA	HS	ACABDA	VII	DBBBBBB	HS	IX
	6	2118	AA	AA	AA	AA	AA	HS	ACABDA	VII	DBBBBBB	HS	IX
	6	2146	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX

TABLE 2. CONTINUED.

Loc	HI	ID	sc nuclear markers					mtDNA type	mtDNA haplotype	mtDNA class	rDNA genotype	rDNA type	rDNA class
			P6	Ldh	Aat	Sod1	Sod2						
	6	2147	AA	AA	AA	AA	AA	HS	ACAADA	VI	BDAAAAA	HSXF6	XII
	6	2148	AA	AA	AA	AA	AA	HS	ACACDA	VIII	BDAAAAA	HSXF6	XII
	6	2149	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2150	AA	AA	AA	AA	AA	HS	ACACDA	VIII	DBBBBBB	HS	IX
	6	2151	AA	AA	AA	AA	AA	HS	ACACDA	VIII	DBBBBBB	HS	IX
	6	2152	AA	AA	AA	AA	AA	HS	ACACDA	VIII	DBBBBBB	HS	IX
	6	2153	AA	AA	AA	AA	AA	HS	CCACDA	IX	DBBBBBB	HS	IX
	6	2154	AA	AA	AA	AA	AA	HS	ACACDA	VIII	DBBBBBB	HS	IX
	6	2155	AA	AA	AA	AA	AA	HS	ACAADA	VI	DBBBBBB	HS	IX
	6	2156	AA	AA	AA	AA	AA	HS	ACAADA	VI	BDAAAAA	HSXF6	XII
	6	2157	AA	AA	AA	AA	AA	HS	ACAADA	VI	BDAAAAA	HSXF6	XII

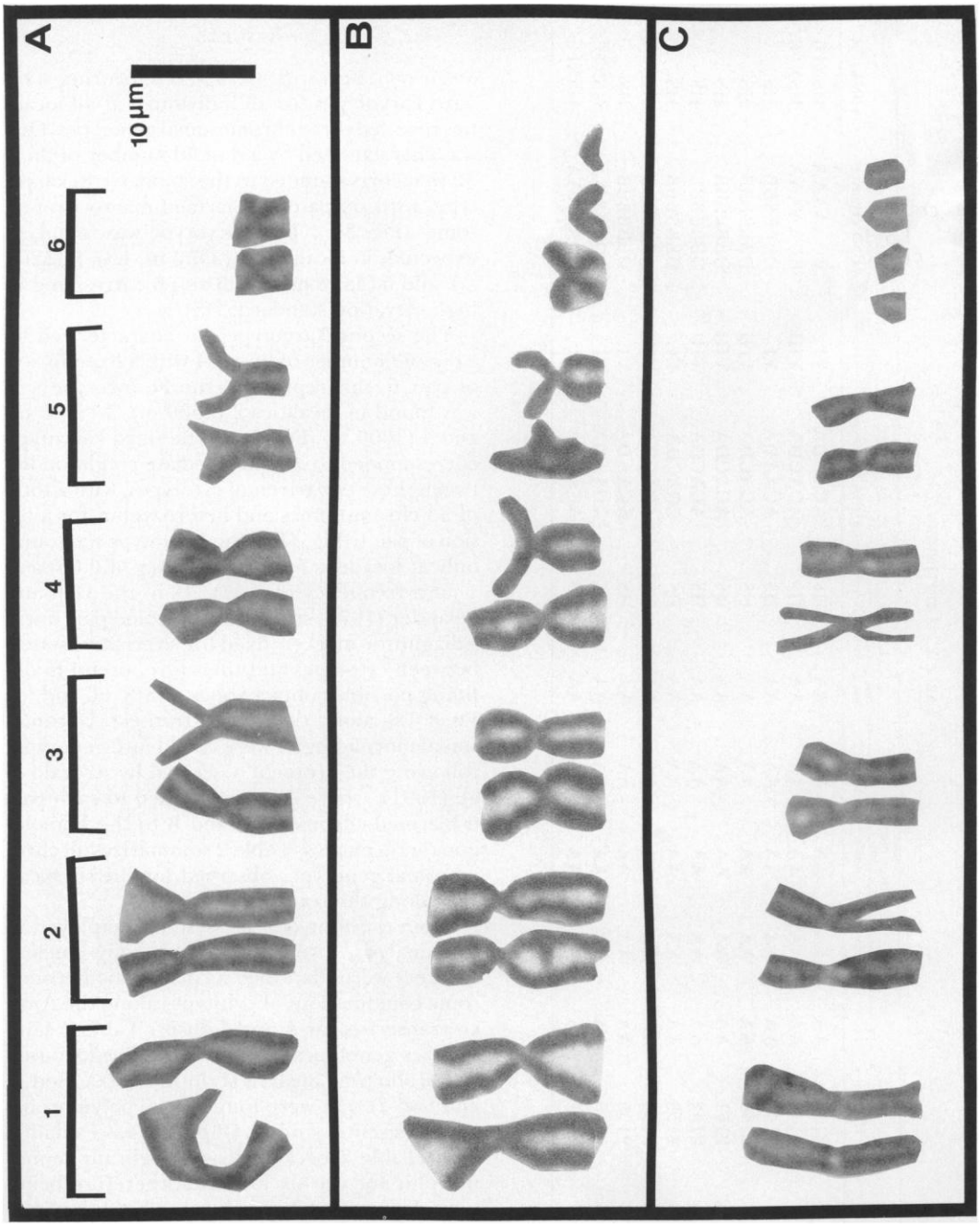
bers of empty cells on the validity of the χ^2 test (Roff and Bentzen, 1989). Our test is based on 1000 randomizations of the original data matrix.

RESULTS

Single-copy nuclear (scn) markers.—Scoring standard karyotypes for all individuals at all localities revealed three chromosomal genotypes. One was characterized by a diploid number of 2n = 32 that corresponded to the standard (S) karyotype, with six pairs of biarmed macrochromosomes (Fig. 3A). This karyotype was found, as expected, in localities 1 (2350 m, LS), 5 (3200 m), and 6 (3350 m, sites 5 and 6 correspond to high elevation standard, HS).

The second karyotype was characterized by a diploid number of 2n = 34 with a fixed fission of pair 6; this represents the F6 cytotype and was found in localities 2 (2600 m), 3 (2800 m) and 4 (3000 m) (Fig. 3C). The third karyotype corresponded to an intermediate condition between these two parental cytotypes, with a total of 33 chromosomes and heterozygous for a fission of pair 6 (Fig. 3B). This karyotype was found only at localities 5 (at a frequency of 0.06) and 1 (at a frequency of 0.07). As in the Hall and Selander (1973) study, chromosome pair 6 was a diagnostic marker (fixed for alternative states) between cytotypes and therefore, useful in defining possible contact zones, HS × F6 and/or F6 × LS, along the Ajusco transect. Chromosomal morphologies were coded into genotypes following the protocol described by Arévalo et al. (1991), where A corresponded to each pair 6 biarmed chromosome and B to the homologous acrocentrics. Table 2 summarizes all chromosomal genotypes observed for the six localities along the transect.

From the four enzyme systems employed in the analysis, two other single-copy nuclear markers were identified as diagnostic between some combinations of cytotypes along the Ajusco transect (sAat-A and Ldh-B). Table 2 summarizes genotypes of four of the five loci used in the allozyme analysis (Ldh-B, sAat-A, Sod-1, and Sod-2) that were found to be polymorphic for the six sites studed; G3pdh-A was excluded from Table 2 because it was allelically monomorphic for the six localities, therefore being noninformative (although it has been duplicated in the F6 cytotype, and is discussed in detail by Sites and Murphy, 1991). The two diagnostic protein markers were fixed for alternative alleles between the HS and F6 races: HS alleles were designated "A" for both loci, and the alternative alleles were designated "B" in F6. The


A
1
2
3
4
5
6
10 µm
B
C

LS race, however, was generally characterized by the same allele as F6 at Ldh-B and shared the same allele as HS at sAat-A (see also Sites et al., 1988). Thus, two diagnostic markers separate LS and F6 (chromosome 6 and sAat-A), whereas three separate F6 from HS (sAat-S, Ldh-B, chromosome 6).

Figure 4 plots the frequency shift of these three markers along the Ajusco transect. This figure shows a sharp, strongly concordant transition between localities 5 and 4 (HS and F6, respectively) for all three markers and a slightly less concordant shift between localities 2 (F6) and 1 (LS) for chromosome 6 and Ldh. Inspection of Figure 4 reveals that the sections of transect inhabited by the HS and F6 cytotypes (sites 5 and 4) are at 3200 and 3000 m, respectively. The physical distance between these two collecting points is approximately 4.0 km, due to the winding road that served as the transect, but the elevational distribution of both cytotypes suggests that the sharp transition for all three markers occurs over a much shorter linear distance. The transition from F6 to LS (between localities 2 and 1) is less distinct, primarily because sAat-A alleles diagnostic of each race are found in the other. In the F6 cytotype, the sAat-A(b) allele is fixed at localities 3 and 4 but segregates with the sAat-A(a) allele (typical of the LS cytotype; Sites et al., 1988) at locality 2 at a frequency of 0.85. At locality 1 the sAat-A(a) allele segregates at a frequency of 0.75 (Table 2). A single animal at locality 1 is heterozygous for the pair 6 fission, whereas none of the metacentric chromosomes are present at localities 2, 3 or 4. There is also no occurrence of F6 alleles at either SOD locus in the LS population (Table 2).

To visualize this transition in terms of proportion of the individual genotypes at each locality, a hybrid index score was calculated for all lizards for chromosome 6 and the two allozymic markers. This index ranged from zero to six, where zero designated an individual with BB genotypes for the three markers, and six consisted of all AA genotypes for the three markers. Using this index, an HS × F6 F_1 individual would have a hybrid index score of 3, because it would by definition be heterozygous at all three diagnostic loci. Backcross individuals

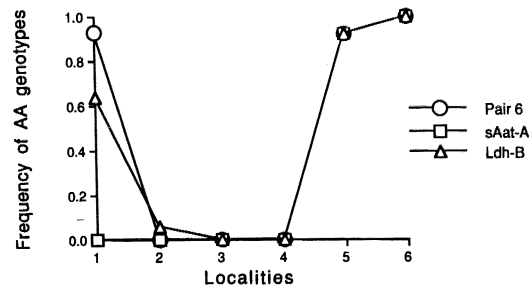


Fig. 4. Distribution of AA genotypes in *Sceloporus grammicus* for three diagnostic markers (as identified in text) by locality along the Ajusco transect. Numbers on horizontal axis correspond to six localities identified in Tables 1 and 2 and in Figure 2.

would have scores between one and five, depending upon the extent of introgression by markers at one or two loci (Table 2). We did not include locality 1 in this comparison, because only two markers (pair 6 and sAat-A) separated LS from F6, and this was insufficient for unambiguous identification of F_1 and backcross individuals.

All individuals (n = 21) at locality 6 and most at locality 5 had scores of six, indicating pure HS genotypes at the three markers. However, two animals from locality 5 were F_1 hybrids. At lower elevations, more individuals correspond to pure F6 genotypes (hybrid index scores = 0) but with some evidence of introgression. At locality 4, for example, two individuals each had hybrid index scores of one (Table 2) and had genotypes characteristic of backcrosses to F6.

Table 3 summarizes pairwise genetic distances among all combinations of localities along the Ajusco transect. The genetic distances within the F6 and HS cytotypes are much smaller (0.000 to 0.011 and 0.003, respectively) than any of the distances between the cytotypes (0.244–0.768). A UPGMA phenogram (Fig. 5A) depicts the clustering of these samples, and, as expected, all cytotypes are clearly distinguished by their allozyme patterns.

Multicopy nuclear markers: rDNA genotypes.—A total of 67 specimens was used as a subsample for the rDNA analysis, and restriction digests with the seven different enzymes revealed a to-

←

Fig. 3. Partial mitotic karyotypes of *Sceloporus grammicus* (macrochromosomes only) taken from Ajusco transect. (A) Metaphase from normal 2n = 32 animal, showing six pairs of biarmed (metacentric or submetacentric) macrochromosomes (IBH6843-3); this configuration is diagnostic for both LS and HS cytotypes. (B) Individual heterozygous for fission at chromosome pair 6 (IBH6841-13). (C) Individual homozygous for pair 6 fission, typical of F6 (2n = 34) cytotype (IBH6836-6).

TABLE 3. MATRIX OF ALLOZYME GENETIC DISTANCES BASED UPON THREE POLYMORPHIC LOCI (BELOW DIAGONAL) AND WEIGHTED mtDNA DISTANCES (ABOVE DIAGONAL) FOR ALL PAIRWISE COMBINATIONS OF *Sceloporus grammicus* SAMPLED ALONG THE AJUSCO TRANSECT.

Locality	1	2	3	4	5	6
1-LS	—	0.054	0.055	0.047	0.072	0.071
2-F6	0.146	—	0.036	0.079	0.086	0.080
3-F6	0.229	0.000	—	0.063	0.085	0.080
4-F6	0.279	0.011	0.000	—	0.051	0.055
5-HS	0.244	0.525	0.655	0 692	—	0.008
6-HS	0.249	0.578	0.725	0.768	0.003	—

tal of 12 different rDNA types across the Ajusco transect (summarized in Table 2). Five of these types characterized the HS cytotype (types VIII–XII), five different types were characteristic of the F6 cytotype (types III–VII), and two types were located at site 1 (types I, II). One of these types corresponded to the LS cytotype (AAAAAAA), and a single individual had a pattern scored as "F6-like" (LS × F6, BCBBBBB), although this exact genotype was not found elsewhere in the transect (Table 2).

Figure 6 shows the frequency shift for the generalized rDNA genotypes across the six localities of the Ajusco Transect. Generalized rDNA genotypes included all of the characteristic classes of each rDNA for each pure chromosomal genotype (LS = class I in Table 2; F6 = classes III–VI and XII; and HS = classes VII and IX–XI), plotted across the transect. From this figure, it can be seen that the HS-type rDNA is not fixed at any locality; it increases from a frequency of 0.80 to 0.84 from locality 6 to 5, drops to 0.10 at locality 4 (class VII), and is absent from the other localities. An F6 type rDNA is present at locality 6 at a frequency of 0.22 (class XII), absent at locality 5, and all other F6 types are present at localities 4, 3 and 2, at frequencies of 0.85, 1.0 and 1.0, respectively. The LS rDNA is almost fixed at locality 1, whereas a second "LS × HS" type (class VIII) is present at locality 5 at a frequency of 0.16, and absent elsewhere.

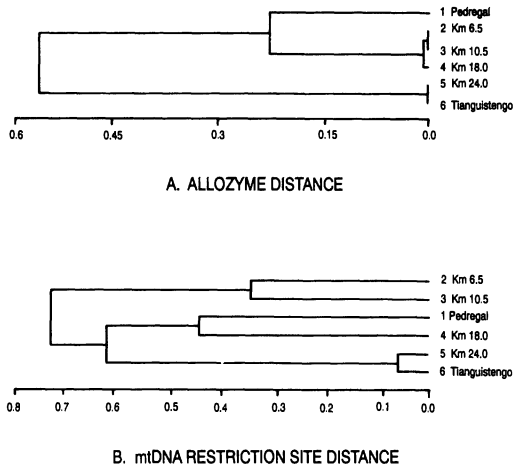


Fig. 5. UPGMA phenograms of genetic similarity for all pairwise comparisons of the six localities for *Sceloporus grammicus* sampled along the Ajusco transect. Phenogram A is derived from the allozyme-based distance values presented below the diagonal in Table 3, whereas B is derived from the mtDNA restriction site-based distances presented above the diagonal in the same table. In both phenograms, numbers are identified by abbreviated localities for the six sites along the Ajusco transect; details are given in Table 1 and plotted in Figure 1.

Cytoplasmic markers: mtDNA haplotypes.—The same subset of 67 lizards was also screened for mtDNA divergence with the same seven restriction enzymes as used in the rDNA analysis. We identified haplotypes from the Ajusco transect on the basis of the presence or absence of mapped restriction sites at the five enzymes that revealed variation in the presence or absence of at least one site between two or more individuals. These five enzymes (Bam HI, Bgl I, Bst EII, Pvu II, and Nco I) revealed a total of nine haplotypes, identified as follows: BAADB, AABAA, BBADC, CABAA, CABAD, ACAAD, ACABD, ACACD, and CCACD. Dra I also revealed variation but did not cut all samples to completion, and Kpn I was invariant. These mtDNA haplotypes are summarized in Table 2. These nine haplotypes were used in all statistical treatments of the Ajusco samples.

Table 4 summarizes evolutionary distances (and standard errors of the estimates) on the basis of mtDNA restriction site differences among all pairwise combinations of the nine haplotypes described above. Distance estimates ranged from a low of 0.009 between haplotypes VI (ACAAD) and VIII (ACACD), which differed by a single Pvu II site, to a high of 0.048 between haplotypes II (BBADC) and IV (CA-

TABLE 4. MATRIX OF EVOLUTIONARY DISTANCES BETWEEN DIFFERENT mtDNA HAPLOTYPES FROM mtDNA RESTRICTION SITE DATA (Nei and Tajima, 1981; Nei and Miller, 1990; below diagonal) AND THEIR STANDARD ERRORS (Nei and Tajima, 1983; Nei, 1987; above diagonal) FOR ALL PAIRWISE COMPARISONS OF NINE HAPLOTYPES OF *Sceloporus grammicus* IDENTIFIED WITH A SUBSET OF FIVE ENZYMES (details in text). Roman numeral class for each haplotype corresponds to those given in Table 2.

	Haplotype	I	III	II	IV	V	VI	VII	VIII	IX
I	BAADB	—	0.037	0.015	0.044	0.041	0.041	0.039	0.033	0.039
III	AABAA	0.085	—	0.041	0.012	0.023	0.023	0.028	0.025	0.037
II	BBADC	0.020	0.101	—	0.048	0.046	0.046	0.044	0.037	0.044
IV	CABAA	0.098	0.016	0.116	—	0.018	0.035	0.044	0.037	0.033
V	CABAD	0.088	0.045	0.107	0.029	—	0.023	0.030	0.026	0.021
VI	ACAAD	0.088	0.045	0.107	0.077	0.042	—	0.010	0.009	0.021
VII	ACABD	0.078	0.059	0.098	0.098	0.058	0.010	—	0.015	0.027
VIII	ACACD	0.068	0.053	0.085	0.085	0.051	0.009	0.019	—	0.015
IX	CCACD	0.078	0.085	0.098	0.068	0.032	0.032	0.048	0.020	—

BAA), which differed by 10 sites (one or more at each enzyme).

MtDNA distance estimates are presented for all pairwise comparisons of localities above the diagonal in Table 3, and Figure 5B presents the UPGMA phenogram derived from this matrix. In contrast to the allozyme-based phenogram (Fig. 5A), the tree of mtDNA d_r values reveals less clear-cut separation of the three chromosome races along the Ajusco transect. Both HS samples (5 and 6) form a distinct group, but the single LS sample is grouped within the F6 samples, because this cytotype is extensively introgressed with F6 mtDNA (Table 2; see also Sites and Davis, 1989). Samples two and three, which are geographically adjacent on the transect, form a second distinct group and are the most divergent samples relative to all others included in this study.

Figure 7 summarizes the frequencies of the three general mtDNA haplotypes across the Ajusco transect. The HS and the F6 types were fixed at the localities of these cytotypes (localities 5 and 6, classes VI–IX; and 2–4, classes III–V, respectively), but a different pattern was found at locality 1 (LS, classes I–III) where F6 or F6-like haplotypes were present at a frequency of 0.55 (class III, see Table 2).

Given the mtDNA diversity within each cytotype apparent in Table 2, the pattern in Figure 7 may not reveal the true pattern of geographic heterogeneity in mtDNA haplotype frequencies. We, therefore, used the nine haplotypes to test for heterogeneity across the Ajusco transect using the Monte Carlo simulations previously described. Table 5 summarizes the distribution of these haplotypes by locality, and the heterogeneity χ^2 value calculated from this original matrix was 156.9. The distribution of the χ^2 values from these simulations was as follows: average $\chi^2 = 40.0$; minimum $\chi^2 = 20.7$, and maximum $\chi^2 = 83.6$. None of the 1000 replicate χ^2 values exceeded that of the original matrix, meaning that the probability of exceeding the original χ^2 by chance alone is very small. The geographic heterogeneity of mtDNA haplotype frequencies summarized in Table 5 is, therefore, highly significant.

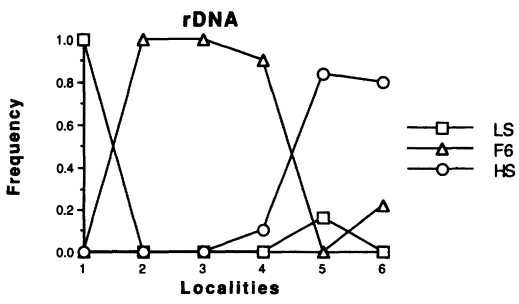


Fig. 6. Distribution of three "general" rDNA types (LS, F6, and HS) in samples of *Sceloporus grammicus* along the Ajusco transect; numbers of each genotype at each locality are summarized in Table 2.

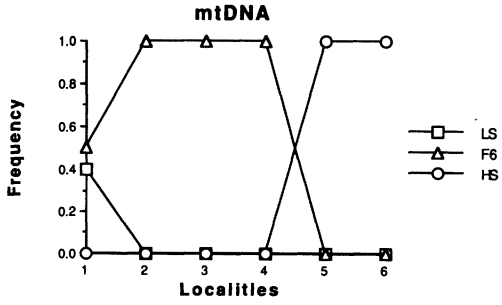


Fig. 7. Distribution of three "general" mtDNA types (LS, F6, and HS) in samples of *Sceloporus grammicus* along the Ajusco transect; symbols are as in Figure 6.

TABLE 5. DISTRIBUTION OF NINE mtDNA HAPLOTYPES IDENTIFIED AMONG SAMPLES OF *Sceloporus grammicus* FROM THE AJUSCO TRANSECT. Roman numerals identify individual haplotypes described in text and summarized in Table 2, and localities are those described in Table 1 and Figure 2.

Locality	I	III	II	IV	V	VI	VII	VIII	IX
1	1	5	3	0	0	0	0	0	0
2	0	8	0	1	0	0	0	0	0
3	0	2	0	1	0	0	0	0	0
4	0	8	0	1	1	0	0	0	0
5	0	0	0	0	0	14	4	0	0
6	0	0	0	0	0	7	3	6	1

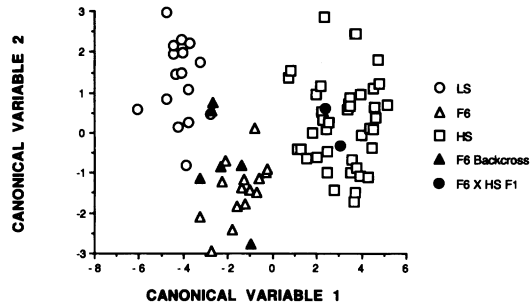


Fig. 8. Canonical discriminate analysis scatter plot of five morphological classes of *Sceloporus grammicus*: LS, F6, HS, F6 backcross, and F_1 hybrids, as defined by hybrid index scores (see Table 2; Fig. 5). The horizontal axis corresponds to the first canonical variable and the vertical axis to the second canonical variable, which ordinate seven meristic characters that maximize separation of parental types. Symbols correspond to open circles = LS, open triangles = F6, squares = HS, closed triangles = F6 backcrosses, and closed circles = F_1 hybrids. Morphological data are included only for animals taken from the Ajusco transect with known chromosome and allozyme characters.

Morphological divergence.—Ten meristic characters were recorded for all animals karyotyped from the Ajusco transect and used for the statistical analysis. The SDA selected seven of the 10 variables used that maximized the separation of these races, including Auricular Lobules (AL), Dorsal Spots (DS), Frontoparietals (FP), Infralabials (IL), Lorilabials (LL), Supralabials (SL), and Supraoculars (SO).

Table 6 summarizes the mean and range for these variables for the six localities across the Ajusco transect. For most of the variables, the means were very similar for the six localities. The sole exception was dorsal spots (DS); the mean values for localities 5 and 6 (HS, 10.7 and 11.0, respectively) were larger than the values for the other two cytotypes (8.0, for localities 1, 2, and 3 and 7.8 for locality 4).

These seven variables were used in a CDA to ordinate all individuals assigned a priori to one of four genotypic classes on the basis of the three diagnostic single-copy nuclear markers. Low Standard individuals were identified as such strictly on the basis of karyotypes (2n = 32) and locality (site 1). For the other two races, all lizards with a hybrid index score of six were classified as pure HS, those with scores of zero were considered pure F6, and all animals with scores ranging from one to five were designated as "hybrids."

Figure 8 shows results of the CDA in which the two canonical discriminant variables describing most of the variability are plotted for

TABLE 6. SUMMARY OF CHARACTERS IDENTIFIED BY A STEPWISE DISCRIMINANT ANALYSIS TO SHOW MAXIMUM DIVERGENCE BETWEEN THE LS, HS, AND F6 CYTOTYPES OF *Sceloporus grammicus* ACROSS THE AJUSCO TRANSECT. Localities are those in Table 1 and Figure 2; and the data summarized include th mean count and ± standard error per sample, and the range below in parentheses.

Locality	n	Auricular lobules	Dorsal spots	Frontoparietals	Intralabials	Lorilabials	Subdigital lamellae	Supraoculars
1	16	10.4 ± 1.5 (3–8)	8.0 ± 0.8 (7–10)	2.5 ± 1.2 (3.0–4.2)	13.5 ± 1.3 (12–15)	25.1 ± 4.4 (15–30)	8.8 ± 0.8 (8–10)	15.1 ± 1.1 (14–17)
2	9	8.9 ± 0.9 (8–10)	8.0 ± 0.9 (6–9)	8.1 ± 1.9 (4.8–9.4)	13.3 ± 1.2 (11–15)	23.8 ± 3.2 (21–28)	8.3 ± 0.7 (8–10)	14.7 ± 1.2 (12–16)
3	2	12 ± 0.0 (12)	8.0 ± 1.4 (7–9)	1.4 ± 0.4 (1.3–1.4)	12.5 ± 0.7 (12–13)	28.0 ± 4.2 (25–31)	8.0 ± 0.0 (8)	15.5 ± 2.1 (14–17)
4	20	9.1 ± 1.7 (6–12)	7.8 ± 0.9 (7–9)	2.0 ± 1.9 (1.7–2.3)	11.2 ± 1.0 (10–13)	25.3 ± 4.0 (21–35)	8 ± 0.5 (7–9)	14.7 ± 1.3 (13–17)
5	31	8.5 ± 1.5 (6–13)	10.7 ± 1.2 (9–13)	2.9 ± 0.4 (2.3–3.5)	12.0 ± 2.2 (9–15)	23.5 ± 4.4 (12–35)	8.5 ± 1.2 (8–13)	14.9 ± 1.1 (13–17)
6	20	9.4 ± 1.1 (8–12)	11.1 ± 1.2 (9–13)	3.9 ± 0.2 (3.5–4.2)	12.2 ± 0.9 (10–13)	21.9 ± 3.1 (17–26)	8.1 ± 0.3 (8–9)	14.7 ± 1.1 (13–17)

all of the individual lizards. The CDA unambiguously separates all pure individuals of the three cytotypes, based on the first canonical variable (Wilks' Lambda = 0.058, df = 14; $P < 0.0001$). The canonical correlation coefficient for this variable is 0.947, and the correlations of the seven characters with this first canonical variable are FP = 0.958, DS = 0.839, AL = −0.333, SL = −0.265, SO = 0.057, LL = −0.319, IL = −0.269. The first two of these characters (FP and DS) contribute most heavily to the separation of the three cytotypes. The second canonical variable shows no additional separation between HS and LS races but further separates all pure F6 individuals. The canonical correlation coefficient for this variable is 0.606, and the correlations of the seven characters with the second canonical variable are SL = 0.553, IL = 0.496, AL = 0.422, DS = 0.344, SO = 0.309, LL = −0.199, and FP = −0.168, much lower values than those provided by the first canonical variable. The two canonical variables together show that the three cytotypes can be distinguished by a combination of dorsal spots, frontoparietals, supralabials, infralabials, and auricular lobules.

The distribution of the F6 backcrosses (see Fig. 8) determined by the three diagnostic markers and provided by the hybrid index score (values 1 and 2) fell mainly within the morphological space of pure F6 individuals. An exception was found in two backcross individuals from locality 2, that in Figure 8 grouped within the distribution of the LS individuals. After considering all the different classes of markers used in this study, we observed that these two individuals (CAP 1413, and 2140) had an allele that was present in both standard cytotypes (LS and HS) for the AAT marker locus (allele A) that was absent in all F6 individuals. Because the distribution of these F6 individuals belonging to locality 2 was closer to locality 1 (LS) than the HS localities (5 and 6), and because they did not show any other markers characteristic of the HS race, we considered that they represent introgressed (LS) alleles from locality 1. All other F6 backcross individuals grouping within the F6 morphospace are from localities 2, 3, and 4 and could represent real HS to F6 backcross combinations. The morphological distribution of the two F_1 individuals from locality 5 (see Fig. 8) was centered within the morphospace of the HS race.

Discussion

Shape of the Ajusco contacts.—Several cytotypes within the *S. grammicus* complex have been found to produce contact zones (Hall, 1980; Sites and Davis, 1989; Arévalo et al., 1991; Fig. 1). In some of these contacts, individuals from the parapatrically distributed cytotypes are known to hybridize and produce F_1 and backcross individuals. Hall and Selander (1973) found a contact zone between individuals of the HS (referred to as the P1 in that paper) and F6 cytotypes along the eastern divide of the Valley of Mexico. They found that the distribution of the F6 cytotype extended from 3000–3400 m in a humid dense oak and pine forest and that the HS was located above this vegetation type up to the tree line (around 4000–4500 m) in an open forest of pine and fir characterized by a ground cover of grass. Below 2600 m, the LS race occupied the floor of the Valley of Mexico. The HS × F6 contact described by Hall and Selander (1973) centered on an ecotone between different vegetation types (humid fir vs open pine). These investigators were able to sample a contact extensively, although this required several independent transects, but all showed that the approximate width of the zone (as inferred from the distribution of F_1 and backcross genotypes) was 400–500 m. Hall and Selander (1973) concluded that no apparent introgression occurred from HS into F6 but that a low level of introgression occurred in the opposite direction. Hall and Selander (1973) also argued that (1) backcross individuals had poor survival; and (2) those that survived to maturity did not reproduce. Hall later (1980) argued that F_1 individuals and the first generation of backcrosses were sufficiently fertile to allow significant introgression beyond the first generation of backcrossing. This contradicted conclusion 2 above, but the evidence unequivocally demonstrated random mating, hybrid fertility, and successful backcrossing within the zone, whereas the zone itself functioned as a complete barrier to introgression in either direction over a distance of about 3 km.

In this study, we confirmed the intermediate distribution of the F6 cytotype between the two races of the "standard" cytotype—LS on the floor of the Valley of Mexico and HS on the highest peaks—on the southwestern side of Mexico City in the Ajusco mountain range. The Ajusco transect revealed evidence for slight introgression of one diagnostic marker from HS into F6 (at Ldh-B in locality 4; see Table 2) but basically showed sharp, concordant shifts among three nuclear markers (chromosome pair 6, sAat-A, and Ldh-B) from HS to F6, over a very short geographic distance (perhaps less than 2 km, compare Figs. 2 and 4). These clines centered near or just below 3000 m (between lo-

calities 4 and 5 in Fig. 2), and two F_1 hybrid individuals were represented in the sample of 31 lizards from locality 5 (Table 2). Very few backcross individuals were collected, however, despite intense efforts on our part, so we were unable to clearly delimit the structure of this contact in detail. The low density of F_1 hybrid and backcross individuals at these localities (two F_1 animals from locality 5, four possible F6-backcrosses at other localities; Table 2) may mean that either hybridization is rare between these two races, or that the population densities in the zone are low. According to hybrid zone theory, contacts such as this should move across the landscape and eventually "settle" either in troughs of low density, or ecotones between adjacent habitats (Barton and Hewitt, 1989; Harrison, 1991; Hewitt, 1988). Based on our collecting efforts, in which greater energy was expended per animal caught in this region (particularly locality 5), our impression is that the density is lower in this part of the transect. There is no obvious ecological transition between localities 4 and 5, because both occur in moderately open, relatively undisturbed pine forest (Table 1). We, therefore, suggest that the HS × F6 contact is situated in a density trough and that low levels of hybridization and backcrossing are sufficient for low frequency introgression of HS markers into the F6 cytotype.

One other feature of the Ajusco transect deserving mention is the evidence for LS × F6 interaction at lower elevations of the transect. We included only a single LS sample in this study because previous studies have shown that it is widely distributed across the floor of the Valley of Mexico (Porter and Sites, 1986; Arévalo et al., 1991) and that it can be characterized as a genetically discrete entity on the basis of several nuclear markers (Sites et al., 1988; Sites and Davis, 1989). Our genetic surveys for this study revealed only two diagnostic markers for the LS and F6 cytotypes—chromosome pair 6 and sAat-A—but one of these (sAat-A) clearly revealed pronounced introgression in both directions; and a single F6 chromosomal marker was present in the LS sample from locality 1 (Table 2). However, both SOD loci were polymorphic and segregated (b) alleles at other localities that were not present in LS. This pattern implies that selection against introgression between these races is marker specific; it appears to be strong against chromosome pair 6, Sod-1, and Sod-2 but less intense at sAat-A (see Fig. 4). We suggest that the LS × F6 contact acts as a semipermeable filter (also see below), and it merits more detailed study.

Patterns of ribosomal-DNA variation in the Ajusco transect.—We found a total of 12 different rDNA types along the Ajusco transect that corresponded to the LS (class I), F6 (II-VI), and HS (VII-XI) cytotypes. However, one of the HS types (class VII) was found at F6 locality 4, and an "HS × F6" type (XII) was found at HS site 6. One of the F6 types (class II) was found in low frequency (one individual, CAP 1471) at LS locality 1; however, no LS rDNA types were found at any of the F6 localities (Table 2). We also found one individual at locality 4 with an HS rDNA type (CAP 1438), but none of the other markers showed any HS introgression. A similar pattern was evident at locality 6, in which four animals with F6 rDNA types (type XII, CAP numbers: 2147, 2148, 2156, and 2157) did not show any other F6 markers. These distributions suggest that rDNA repeats may simply flow freely along this transect or that novel patterns are generated within populations by recombination within rDNA repeats. Larger sample sizes will be needed to assess these alternatives.

Levels of divergence and taxonomic implications.—Grouping localities by mtDNA divergence estimates (Table 4, Fig. 5B) showed that the separation of the HS race was clear; however, LS did not clearly separate from F6. This is undoubtedly due to extreme asymmetrical introgression of F6 mtDNA (types III) into the LS cytotype (Table 2, and Fig. 7). In a more general geographic survey, Sites and Davis (1989) also found extensive mtDNA introgression from the F6 race into LS but not the reverse.

There are several possible explanations for the asymmetrical pattern of introgression between F6 and LS cytotypes. An obvious explanation is that assortative mating may occur in which female F6 animals preferentially choose to mate with LS males. A behavioral study by S. Moody (pers. comm.) between the HS and F6 races hybridizing in the Río Frio transect described by Hall and Selander (1973) demonstrated strong potential for asymmetry in female choice. Moody (pers. comm.) has demonstrated that HS females selectively associated more often with F6 males, larger than the HS males, given a choice between adult males of both cytotypes. In the reciprocal test, F6 females associated with F6 males more frequently than with HS males. If similar associations occur between the LS and the F6 races, and if such associations lead to significant levels of nonrandom mating, an asymmetrical bias in mitochondrial flow would be established. In this part of

Mexico, the LS males are much larger than F6 males, and preferential mating by F6 females more often with LS males would maintain an asymmetrical flow of F6 mtDNA into the LS cytotype.

Two alternative explanations are possible: (1) random mating may occur and be coupled with strong selection against LS mtDNA on an F6 nuclear background (Moritz et al., 1987); or (2) the F6 mtDNA represents an ancestral polymorphism that has subsequently become fixed in the F6 race after the divergence of the two cytotypes (Moritz et al., 1987; Avise, 1989; Sites and Davis, 1989). We cannot rule out either of these alternatives and emphasize that they are not mutually exclusive. However, all the cytotypes that have been surveyed segregate for extensive mtDNA haplotype diversity (Sites and Davis, 1989; Sites et al., 1993), so explanations involving ancestral polymorphisms could account for almost any conceivable pattern in the absence of a robust phylogenetic hypothesis derived independent of an mtDNA data base. Selection against LS mtDNA introgression into an F6 background is also certainly plausible, but such explanations require careful elimination of many other possibilities (Forbes and Allendorf, 1991), which we cannot do with the data presented here.

With one exception, the morphological data did not seem to provide clear markers for the different cytotypes when analyzed on a single character basis, but the number of dorsal spots (DS) increased with elevation (Table 6). The SDA analysis provided a clear separation of the three cytotypes (Wilks lambda = 0.058, df = 14; $P < 0.0001$), so that the genotypically "pure" individuals behaved as discrete entities. The morphological phenotypes of the hybrid and backcross individuals were included within one of the three genotypically pure morphological groups. Two of the backcrossed individuals from locality 2 were grouped within the distribution of the LS individuals, and according to one of their diagnostic markers, sAat-A, both were introgressed with LS alleles. Hybridization between LS and F6 and subsequent backcrossing might produce phenotypes skewed in the direction of backcrossing, possibly explaining the location of these animals in the LS morphospace. The morphological distribution of the two F_1 individuals (see Fig. 8) was not intermediate between the F6 and HS morphospace; it was centered within the morphospace of the HS race. Four other lizards had hybrid index scores of one or two, and were collected either from localities 2 or 4 (Table 2). All, however grouped in the F6 morphospace, even though animals from the locality 2 were likely introgressed with LS alleles, whereas those from locality 4 were probably introgressed with HS alleles. The distribution of these four lizards in Figure 8 suggests that the morphological traits sampled in this study are not clearly indicative of the genetic makeup of animals of mixed parentage. Individual morphological traits are likely controlled by one or a few loci in which allelic dominance has morphological consequences, or controlled by complex epistatic, polygenic systems (Ross and Cavender, 1981). The outcome of hybridization yields a range of morphologies, few of which are precisely intermediate.

Several studies have been carried out for these three cytotypes, each giving contrasting relationships between them. Hall and Selander (1973) reported hybridization at a contact zone between HS and F6, and asymmetrical introgression (based on allozymes) was observed from HS to F6. They did not consider the introgression to affect the genetic integrity of these cytotypes and concluded that the hybridizing populations were distinct biological species. More recently, Lara (1983) recognized the LS, HS, and F6 cytotypes as distinct biological species on the basis of a combination of four or five characters. He assigned the LS population to the name *S. g. microlepidotus* and named *S. anahuacus* (HS) and *S. palaciosi* (F6) as new species. An allozyme study by Sites et al. (1988) supported the recognition of F6 (*S. palaciosi*) as a biological species based on several allozyme markers. In contrast, the genetic distinction between the two standard cytotypes (LS and HS) was based on a single fixed allozyme marker (of 38 loci resolved), leading Sites et al. (1988) to question the specific status of HS (*S. anahuacus*). Sites and Davis (1989) analyzed restriction site variation in both mtDNA and rDNAs within and between seven of the eight cytotypes of *S. grammicus* known from central Mexico and showed that three samples of the HS cytotype formed a monophyletic group supported by seven rDNA restriction sites (six gains and one loss). This same clade was further supported by 10 mtDNA restriction sites (seven gains and three losses), making it the most well-defined group of any identified by Sites and Davis (1989).

These conflicting conclusions stem from at least two sources. First, none of the molecular studies to date have included more than a single sample of the F6 race (the same specimens were used for the allozyme and DNA studies by Sites et al., 1988; and Sites and Davis, 1989), even though it was originally described from several mountain ranges (Lara, 1983). Similar limitations hold for the LS populations, although

studies now in progress include multiple localities for all of these races. Second, the initial description of *S. anahuacus* for the HS samples was based on morphological characters, whereas the conservative conclusion of Sites et al. (1988) was based on the inability to characterize the single fixed allozyme marker for some of these same samples in a phylogenetic context. A decision regarding the specific status of any of these chromosome races will depend to a great extent on whether one chooses to emphasize the hybridization or phylogenetic aspects of these investigations. There is certainly enough hybridization and backcrossing between parapatrically distributed forms to allow exchange of some nuclear and mitochondrial markers, although this usually appears to be limited to areas immediately adjacent to the zones (Hall and Selander, 1973; Sites et al., 1993; this study). Some investigators would argue that hybridization may occur due to the retention of ancestral characters (ability to interbreed) and that speciation is not complete if there is not clear evidence for selection against hybrids (e.g., Dessauer and Cole, 1991). Alternative views emphasize the diagnosis of independently evolving lineages by one or more synapomorphic characters recovered in phylogenetic studies (reviewed by Cracraft, 1987; Frost and Hillis, 1990; Nixon and Wheeler, 1990). In our opinion, these views of evolutionary or phylogenetic species concepts are internally consistent and provide robust methods for defining species provided that sufficient sampling efforts are carried out. These approaches are not problem free at very low hierarchical levels (de Queiroz and Donoghue, 1990), but in the case of the populations designated by Lara (1983) as *S. anahuacus* and *S. palaciosi*, we believe that enough synapomorphic characters are or soon will be available for an adequate series of samples to show that they are independent lineages (Sites and Davis, 1989; EA, SKD, JWS, unpubl. data). Similar decisions regarding the other central Mexico chromosome races of *S. grammicus* must await completion of ongoing population genetic and phylogenetic studies.

Origin and maintenance of the Ajusco contacts.— Limited phylogenetic information available from the previous study by Sites and Davis (1989) show the HS and F6 races as nonsister taxa on the basis of mtDNA restriction sites and as an unresolved basal polytomy with all other races on the basis of rDNA sites. If the nonsister relationship is verified, then the contact between these two can be inferred to have a secondary origin. This interpretation is consistent with the data presented in this paper and by Hall and Selander (1973), showing a concordant shift for the three diagnostic markers as well as for mtDNA haplotypes. Further, the overall levels of divergence among the three races across the three classes of molecular markers (allozymes, mtDNA, and rDNA) and morphology (Fig. 8) suggest that all three cytotypes have diverged in allopatry and that both contacts are secondary in origin. Hall and Selander (1973) claimed that HS and F6 must have been isolated for at least 7000 years, on the basis of palynological data from central Mexico, and have only recently come into secondary contact. Our estimates of divergence would imply a much older isolation time (Table 3; Fig. 5), making a primary origin hypothesis for the LS × F6 contact very unlikely, unless we postulate that all independent concordant markers are responding to the same selection gradient in the same manner. We do not think this plausible and conclude that both contacts along the Ajusco transect are of secondary origin.

We do not have data bearing directly on maintenance of either the F6 × LS or F6 × HS contacts, but such contacts may be maintained by a balance between dispersal and intrinsic inferiority of hybrid and/or backcross individuals. Hybrid inferiority may, in turn, result from a number of causes, including direct selection against particular loci in heterozygous combinations (such as chromosomal rearrangements), selection due to epistatic interactions among different combinations of genomes, or frequency-dependent selection against rare alleles (reviewed by Harrison, 1991). Detailed studies on a smaller geographic scale, where lizard densities are high enough, would provide the information needed to resolve these issues.

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(EA, JWS) DEPARTMENT OF ZOOLOGY, BRIGHAM YOUNG UNIVERSITY, PROVO, UTAH 84602; (GC) LABORATORIO DE HERPETOLOGÍA, INSTITUTO DE BIOLOGÍA, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO, MEXICO 04510; (SKD) DEPARTMENT OF ANIMAL SCIENCE, TEXAS A & M UNIVERSITY, COLLEGE STATION, TEXAS 77843; (GL) CENTRO DE ECOLÓGICO DE SONORA AP. POSTAL 1497, HERMOSILLO, SONORA, MEXICO. Submitted 8 Nov. 1991. Accepted 9 March 1992. Section editor: D. G. Buth.