Copeia, 1993(2), pp. 373–398

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

JACK W. SITES, JR., SCOTT K. DAVIS, DELBERT W. HUTCHISON, BRIAN A. MAURER, AND GUILLERMO LARA

Genetic and morphological studies were carried out on a total sample of 108 individuals of the *Sceloporus grammicus* complex, collected from eight sites along a transect in eastern Hidalgo, Mexico. Samples were collected from both sides and through the middle of a zone of parapatric hybridization between F5 (2n = 34) and FM2 (2n = 46) chromosome races of this group to assess general patterns of gene flow across the zone for independent sets of markers. Karyotypic and morphological data were collected from almost all lizards; and allozyme, mitochondrial-DNA, and ribosomal-DNA restriction site data were collected from a subset of the total. Three unambiguous diagnostic chromosome markers defined the zone by showing sharp, concordant changes from one parental race to the other over a distance of about 2 km. Virtually no introgression of any of these markers occurred outside of localities immediately adjacent to the point of contact, and this transition was also reflected in diagnostic classes of mtDNA haplotypes, albeit slightly asymmetrical in the direction of F5 introgression. Ribosomal-DNA genotypes introgressed more extensively in both directions. No fixed genic differences were found between these cytotypes in a sample of 42 loci, but allozyme frequencies in five polymorphic loci displayed frequency shifts across the transect, as did the distribution of mtDNA haplotypes. Morphologically, chromosomally pure individuals in the zone could be correctly assigned to their appropriate parental populations outside of the zone with a high degree of confidence. F_1 and backcross individuals were morphologically intermediate in multivariate analyses, although they overlapped with both groups of parental phenotypes, and very few single traits displayed intermediacy. The structure and shape of the diagnostic character clines are discussed with respect to the origin and maintenance of this zone.

Se realizaron estudios genéticos y morfológicos en un total de 108 individuos del complejo *Sceloporus grammicus* colectados en ocho sitios a lo largo de un transecto en el este de Hidalgo, México. Las muestras fueron colectadas a ambos lados y a través de la mitad de una zona de hibridización parapátrica entre las razas cromosómicas de F5 (2n = 34) y FM2 (2n = 46) de este grupo, para establecer el patron general de flujo genético a través de la zona por medio de series independientes de marcadores. Se colectaron datos cariotípicos y morfológicos de al mayoría de las lagartijas, asimismo datos alozimáticos, de sitios de restriction del ADN-mitocondrial y de ADN-ribosomal de un subgrupo del total. Tres marcadores cromosómicos diagnósticos unambiguos definieron a la zona mostrando marcados cambios concordantes de una raza parental a la otra, en una distancia de circa de 2 km. Casi no existió introgresión en ninguno de estas marcadores fuera de las localidades se encuentran adjacentes inmediatamente al punto de contacto, esta transición fue tambien reflejada en clases diagnósticas de haplotipos de ADN-mitocondrial aunque un poco asimétrico en la dirección de la introgresión de F5. La introgresión de los genotipos del ADN-ribosomal fue mas extensa en ambos direcciones. No se encontraron diferencias genéticas fijas entre estos citotipos en una muestra de 42 loci, pero las frecuencias alozimáticas en cinco loci polimorficos mostraron cambios en las frecuencias a través del transecto, como sucedió con la distribucción de los frecuencias de haplotipos de ADN-mitocondrial. Morfológicamente, los individuos puros cromosomicamente en la zona pudieron ser asignados correctamente hacia la población parental apropriada fuera de la zona con un alto grado de confianza. Los individuos

F_1 y retrocruzas fueron intermedios morfológicamente en analises multivariados, aunque existió sobrelapamiento en ambos grupos de fenotipos parentales, y muy pocos caracteres unicos demostraron ser intermedios. La estructura y forma de los caracteres clinales se discutieron con relación al origen y mantenimiento de esta zona.

THIS paper, a companion paper to Arévalo et al. (1993), reports on a genetic analysis of a second transect between two cytotypes of the *Sceloporus grammicus* complex in central Mexico, which will be referred to as the Tulancingo transect (arrow B in fig. 1 of Arévalo et al., 1993). The cytotypes involved in this contact include F5, characterized by a 2n = 34 karyotype, and FM2, characterized by a 2n = 44–46 karyotype. In the F5 karyotype, all macrochromosomal pairs except number 5 possess the metacentric [pairs 1, 3, 4 (although some populations segregate for an inversion polymorphism at this pair), and 6] or submetacentric (pair 2) morphology. All macrochromosomes in the FM2 karyotype are fissioned except pair 4 (which segregates for a fission polymorphism), and the complement has an extra pair of microchromosomes (see details in Arévalo et al., 1991). Because these two races differ by at least five fixed chromosome rearrangements involving a substantial portion of the genome, this hybrid zone would be expected to provide a significant postmating barrier to gene flow under the assumptions of underdominance in chromosomal heterozygotes (Walsh, 1982; Sites and Moritz, 1987), recombination effects (Shaw, 1981), or adaptations to alternative ecological conditions (Barton, 1983; Shaw et al., 1990). The immediate objectives of this study were to (1) qualitatively assess the structure of this hybrid zone with respect to cline shape for a number of independent nuclear (single-copy and multicopy) and mitochondrial markers; (2) assess the extent to which morphological divergence reflects the genetic structure of hybridization between these two cytotypes (Neff and Smith, 1979; Dowling et al., 1989); and (3) compare the general patterns of gene flow across this zone in contrast with the Ajusco transect in which the hybridizing cytotypes differed by only a single fixed chromosomal rearrangement (Arevalo et al., 1993).

Materials and Methods

Field sampling.—During the summers of 1985 and 1986, 146 lizards were collected from eight localities along a 23-km transect in the Mexican state of Hidalgo. The transect originated in the town of Tulancingo and extended west along Mexico Hwy 130 toward the city of Pachuca.

Table 1. Localities (Numbered to Correspond to Sites Identified in Fig. 1), Sample Sizes (n) Cytotype (2n), Vegetation Type, and Museum Voucher Numbers for the Eight Localities of *Sceloporus grammicus* Sampled along the Tulancingo Transect. Numbers in parentheses below sample sizes in column n are sample sizes for DNA markers.

Locality	n	2n	Vegetation type	Voucher numbers
1. City of Tulancingo	32 (10)	F5[a] (34)	*Opuntia*, rock walls	EDHEM 1790–1808, 2006–2017
2. 8.2 km W Tulancingo and, 3. 10.0 km W Tulancingo	20 (14)	F5 (34)	Disturbed oak-pine forest, *Agave*	CAP 1958–60, MZFC 1982 (A–P), 1983
4. 400–600 m ENE El Sabino	14 (10)	mixed	Disturbed oak forest, *Agave*, rock walls	CAP 2029–33, 2035–42
5. El Sabino, 11.1 km W Tulancingo	14 (14)	mixed	Disturbed oak forest, *Agave*, rock walls	CAP 2002–06, 2008–09, 11–15, 2027–28
6. La Lagunilla, 12.5 km W Tulancingo	12 (3)	FM2 (44–46)	Isolated trees, *Agave*, rock walls	CAP 1938–40, 62–64, 67–71
7. Matias Rodriguez, 18.8 km W Tulancingo; and 8. 20.3 and 23.3 km W Tulancingo	18 (1)	FM2 (44–46)	Rows of *Agave*	EDHEM 2026, 2039–2040, 2080

[a] The F5 race segregates for a pair 4 inversion polymorphism in some populations, but these are not explicitly dealt with here; see Arévalo et al. (1991) for details.

TABLE 2. ENZYMES AND ELECTROPHORETIC CONDITIONS USED IN ALLOZYME SURVEYS OF *Sceloporus grammicus* SAMPLES COLLECTED FROM THE TULANCINGO TRANSECT. Names and enzyme commission (E.C.) numbers follow recommendations of the Nomenclature Committee of the International Union of Biochemistry (1984); locus abbreviations follow Buth and Murphy (1990); m- and s- prefixes indicate mitochondrial and supernatant loci, respectively; and tissues are designated as: K—kidney, L—liver, and M—skeletal muscle.

Enzyme	E.C. number	Locus	Tissue	Buffer[a]
β-N-acetylgalactosanimidase	3.2.1.53	βGala-1	L	A
		βGala-2	L	A
N-acetyl-β-glucosaminidase	3.2.1.30	β-Ga	L	I
Aconitate hydratase	4.2.1.3	mAcoh-A	L	B
		sAcoh-A	L	B
Alcohol dehydrogenase	1.1.1.1	Adh-A	L	B
Aspartate aminotransferase	2.6.1.1	m-Aat-A	M	C
		s-Aat-A	M	C
Creatine kinase	2.7.3.2	Ck-A	M	B
Dihydrolipoamide dehydrogenase	1.8.1.4	Ddh-A	L	E
Dipeptidases[b]	3.4.13.11	Pep-A	M	D
		Pep-E	M	D
		Pep-F	M	D
		Pep-S	M	D
Fructose biphosphatase	3.1.3.11	Fbp-1	K	C
		Fbp-2	K	C
Fumarate hydratase	4.2.1.2	Fum-A	M	F
General protein	—	Gp-1	L	E
Glucose-6-phosphate isomerase	5.3.1.9	Gpi-A	L	B
αglucosidase	3.2.1.20	αGlus-A	L	A
βglucuronidase	3.2.1.31	βGlur-A	M	G
Glutamate-ammonia ligase	6.3.1.2	Glal-A	L	H
Glutathione reductase	1.6.4.2	Gr-1	L	I
		Gr-2	L	I
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3pdh-A	L	F
L-iditol dehydrogenase	1.1.1.14	Iddh-A	L	F
Isocitrate dehydrogenase	1.1.1.42	Icdh-1	L	B
L-lactate dehydrogenase	1.1.1.27	Ldh-A	L	B
		Ldh-B	L	B
Malate dehydrogenase (NAD+)	1.1.1.37	mMdh-A	L	B
		sMdh-A	L	B
Malate dehydrogenase (NADP+)	1.1.1.40	Mdhp-1	L	B
Mannose-6-phosphate isomerase	5.3.1.8	Mpi-A	L	B
αmannosidase	3.2.1.24	αMan-A	L	E
Phosphoglucomutase	5.4.2.2	Pgm-A	L	B
Phosphogluconate dehydrogenase	1.1.1.44	Pgdh-A	L	F
Phosphoglycerate kinase	2.7.2.3	Pgk-A	M	F
Purine-nucleoside phosphorylase	2.4.2.1	Pnp-A	L	A
Superoxide dismutase	1.15.1.1	mSod-A	L	H
		sSod-A[1]	L	H
		sSod-A[2]	L	H
Triose-phosphate isomerase	5.3.1.1	Tpi-A	M	C

[a] A: Lithium-borate/tris-citrate (250 V, 14–15 h); B: Tris-citrate pH 8.0 (35 ma, 20–24 h); C: Tris-borate-EDTA-I (25 ma, 18–20 h); D: Tris-HCl (250 V, 7–8 h); E: Borate-discontinuous (35 ma, 16–17 h); F: Tris-citrate pH 7.0 (75 ma, 7–8 h); G: Tris-HCl (250 V, 12 h); H: Tris-borate-EDTA-I (250 V, 15–16 h); I: Tris-HCl (250 V, 20–22 h); all buffers are from Buth and Murphy (1990).

[b] Dipeptidase substrates were: glycyl-L-leucine (Pep-A, -S); L-leucine-β-naphthylamide HCl (Pep-E); and L-leucyl-L-leucyl-L-leucine (Pep-F).

In contrast to the Ajusco transect, which passes along a steep forested elevational gradient on the northeast slope of the Sierra del Ajusco range (figs. 1–2 in Arévalo et al., 1993), the Tulancingo transect passed through a disturbed agricultural area across the floor of a small valley. Figure 1 shows the sampling points along the highway; and Table 1 summarizes sample sizes, ecological details, and voucher specimens for each locality.

Lizards were collected and processed for morphological, karyotypic, and molecular data

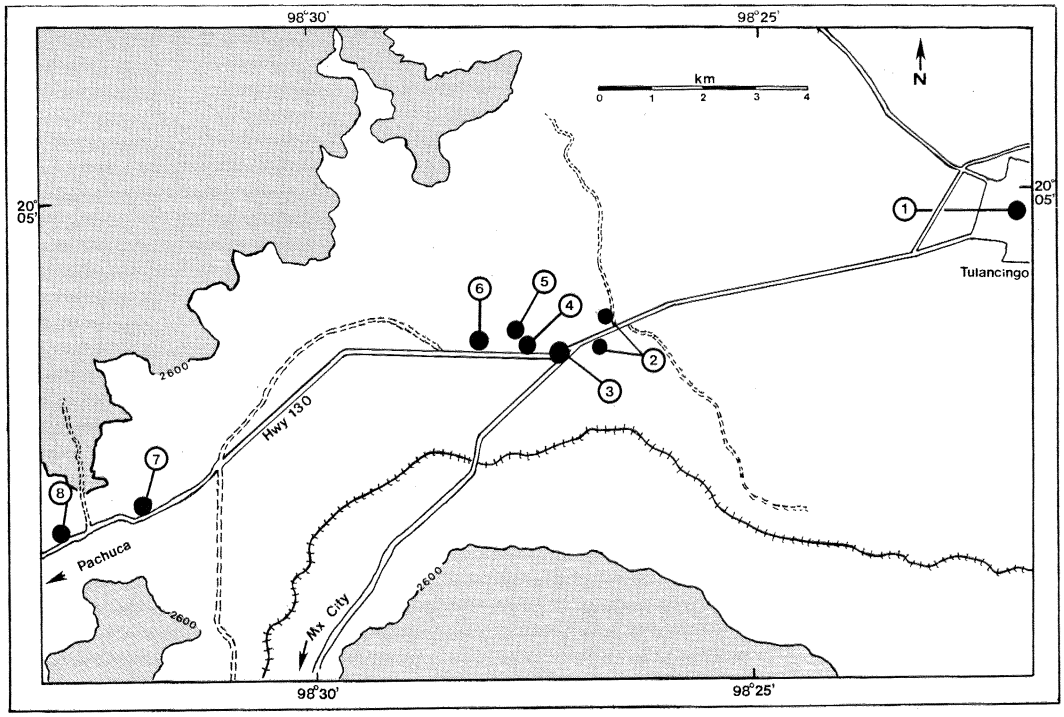

Fig. 1. Map of Tulancingo transect in east-central Hidalgo, Mexico (see hybrid zone B in fig. 1 of Arévalo et al., 1993). Numbers identify collecting localities for *Sceloporus grammicus* summarized in Table 1.

as described in the companion paper by Arevalo et al. (1993). Some individuals, killed in capture, did not provide chromosomal or molecular data. Other lizards were too small to yield enough tissue for all molecular analyses. Morphological data, however, were collected from most specimens, which were prepared as vouchers and catalogued into one of the following collections: Museo de Zoologia–Facultad de Ciencias (MZFC), at the Universidad Nacional Autónoma de México, or the Ecologia de la Herpetofauna del Estado de México collection (EDHEM), at the Escuela Nacional de Estudios Profesionales-Iztacala, UNAM. The CAP prefix for some animals refers to individual field catalog numbers of C. A. Porter for specimens either catalogued as series in single lots (i.e., several specimens included under a single number) or, in some cases for which no voucher was retained, because molecular techniques required use of the entire animal (small juveniles).

Data collection.—Morphological, chromosomal, and molecular data were collected and analyzed as described by Arévalo et al. (1993). Because the F5 cytotype has not been previously included in earlier allozyme or molecular studies (Sites and Greenbaum, 1983; Sites et al., 1988; Sites

→

Fig. 2. Mitotic karyotypes of *Sceloporus grammicus* taken from the Tulancingo transect; microchromosomal pairs are included only for typical F5 (panel A, CAP 1600) and FM2 (panel P, CAP 1609) females, and the partially trisomic individual in panel H (CAP 2635). Others are as follows: B, pair 4 pericentric inversion homozygote (CAP 2059); C, pair 4 pericentric inversion/fission heterozygote (CAP 1820); D, double fission heterozygote (1, 4) CAP 2029); E, double fission heterozygote (3, 4) (CAP 2040); F, fission heterozygote (2) and homozygote (1) (CAP 2032); G, double fission homozygote (2, 6) (CAP 2015); I, F_1-like, fission heterozygote at pairs 1, 2, 6, and 3 (CAP 2005); J, F_1-like, fission heterozygote at markers only (CAP 2012); K, double fission homozygote (2, 6) and pair 1 heterozygote (CAP 2027); L, double fission heterozygote (4, 6) (CAP 2028); M, triple fission heterozygote (1, 4, 6) and pair 3 homozygote (CAP 2006); N, triple fission heterozygote (3, 4, 6) (CAP 2006); O, double fission heterozygote (4, 6) and triple homozygote (1, 2, 3) (CAP 2003); P, double fission heterozygote (3, 4) and triple homozygote (1, 2, 6) (CAP 1938). All karyotypes are magnified to the same scale, which is 10 u in panel A.

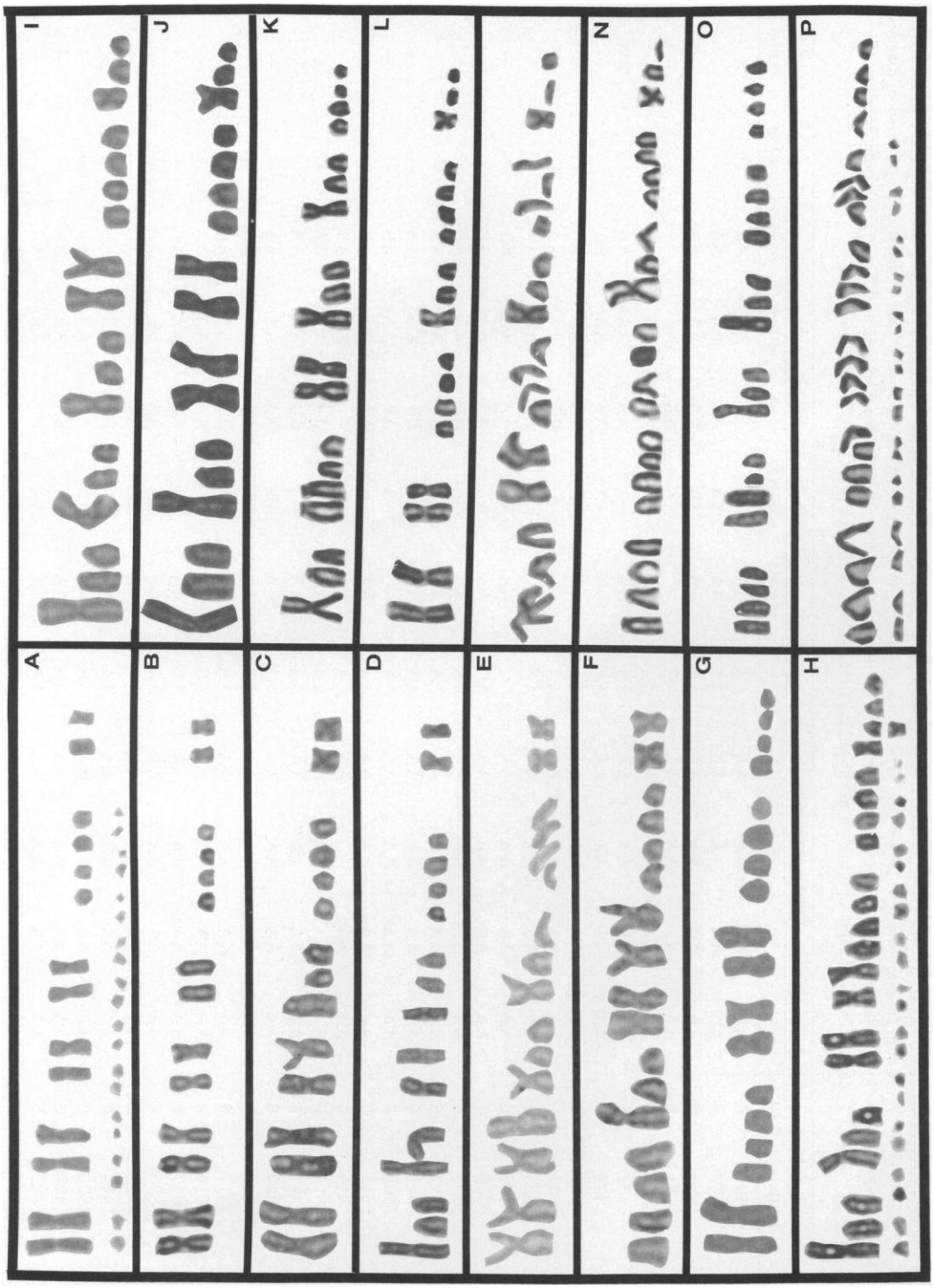
A
B
C
D
E
F
G
H
I
J
K
L
N
O
P

TABLE 3. VARIATION IN GENETIC MARKERS IN SAMPLES OF *Sceloporus grammicus* ACROSS THE TULANCINGO TRANSECT. Sample numbers refer to localities in Table 1 and Figure 1; ID = museum catalog number of each genotype, two individuals from locality 5 identified by "*" are F1 hybrids on the basis of diagnostic chromosome markers; HI = hybrid index score, based on three diagnostic chromosome pairs (bold faced) (see text and Fig. 4); allozyme loci are the five polymorphic markers identified in the text; mtDNA haplotypes are presented in detail in Table 5.

			Chromosome markers					Allozyme loci						
Loc	ID	HI	P1	P2	P6	P3	P4	sAcoh-A	Iddh-A	Ldh-B	αMan-A	Pnp-A	mtDNA	rDNA
1	1599	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	—	AA	AA	AB	F5	A
	1600	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AB	AA	AA	AA	—	—
	1611	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AB	AA	AA	AA	—	—
	1601	6	**AA**	**AA**	**AA**	**AA**	**AC**	—	AA	AB	AA	—	—	—
	1614	6	**AA**	**AA**	**AA**	**AA**	**AA**	—	—	AB	AA	AB	—	—
	1604	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	—	AB	AA	AB	F5	A + B
	1828	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AA	AB	AB	BB	—	—
	1606	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AA	AA	AA	AB	F5*	A
	1607	6	**AA**	**AA**	**AA**	**AA**	**AA**	—	—	BB	AA	AA	F5	A
	1603	6	**AA**	**AA**	**AA**	**AA**	**AA**	—	AA	AB	AA	AA	F5*	A
	1609	6	**AA**	**AA**	**AA**	**AA**	**AC**	AA	AA	AA	AA	BB	—	—
	1610	6	**AA**	**AA**	**AA**	**AA**	**AC**	AA	AA	AA	AA	AB	—	—
	1605	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AA	BB	AA	AB	F5*	A
	1612	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AB	BB	AA	AA	—	—
	1613	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AB	BB	AA	AA	—	—
	1608	6	**AA**	**AA**	**AA**	**AA**	**AA**	—	AB	AB	AA	AB	—	—
	1615	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AB	AA	AA	—	—	—
	1816	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AA	—	AA	AB	—	—
	1818	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AA	AB	AA	AA	F5	A
	1819	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	—	BB	AA	AA	—	—
	1820	6	**AA**	**AA**	**AA**	**AA**	**BC**	AA	BB	AA	AA	AA	—	—
	1821	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	BB	AB	AA	AA	—	—
	1817	6	**AA**	**AA**	**AA**	**AA**	**AA**	AB	AB	AA	AA	AB	F5*	A
	1616	6	**AA**	**AA**	**AA**	**AA**	**AC**	AA	AA	AA	AA	AA	—	—
	1617	6	**AA**	**AA**	**AA**	**AA**	**AC**	AA	AB	AB	AA	AB	—	—
	1826	6	**AA**	**AA**	**AA**	**AA**	**AA**	—	AA	AB	BB	AA	—	—
	1823	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	BB	BB	AA	AB	—	—
	1824	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	—	BB	AB	AB	F5*	A
	1822	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AB	AA	AA	AB	—	—
	1827	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	—	BB	AB	AA	—	—
	1825	6	**AA**	**AA**	**AA**	**AA**	**AA**	AA	AA	AA	AB	AB	—	—
	1602	6	—	—	—	—	—	AB	AB	AA	AA	AA	F5	A

TABLE 3. CONTINUED.

			Chromosome markers					Allozyme loci						
Loc	ID	HI	P1	P2	P6	P3	P4	sAcoh-A	Iddh-A	Ldh-B	αMan-A	Pnp-A	mtDNA	rDNA
2, 3	1958	6	AA	AA	AA	AA	AB	AA	AA	BB	AA	BB	F5	B
	1959	6	AA	AA	AA	AA	AA	AA	AA	AB	AA	AA	F5	B
	2053	6	AA	AA	AA	AA	AA	AA	AA	AB	AA	AA	F5	A
	1960	6	AA	AA	AA	AA	AB	AA	—	BB	AA	AB	F5	A + B
	2043	6	AA	AA	AA	AA	AA	AA	AA	AB	AA	AB	F5	—
	2044	6	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	—	—
	2054	6	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	F5	A + B
	2045	6	AA	AA	AA	AA	AA	AA	—	AA	AA	AA	—	—
	2046	6	AA	AA	AA	AA	AC	AA	AA	BB	AA	AB	F5	B
	2047	6	AA	AA	AA	AA	AA	AA	AA	AB	AA	AB	—	—
	2048	6	AA	AA	AA	AA	AA	AA	AA	AB	AA	AB	F5	A + B
	2049	6	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	F5	A + B
	2050	6	AA	AA	AA	AA	AA	AB	AA	AB	AA	AB	F5	A + B
	2051	6	AA	AA	AA	AA	AA	AA	AA	AB	AA	BB	F5	B
	2056	6	AA	AA	AA	AA	AC	AA	—	AA	AA	AA	F5	B
	2057	6	AA	AA	AA	AA	AC	AA	AA	AA	AA	AB	F5	B
	2059	5	AA	AB	AA	AA	CC	AB	AA	BB	AA	AB	F5	B
	2060	6	AA	AA	AA	AA	AC	AB	AA	AB	AA	AB	F5	B
4	2041	6	AA	AA	AA	AA	AA	AA	AA	BB	AA	AB	F5	B
	2039	6	AA	AA	AA	AA	AA	AB	AA	BB	AA	AB	—	—
	2042	5	AB	AA	AA	AA	AA	AA	AA	AA	AA	AA	—	—
	2030	6	AA	AA	AA	AA	AC	AA	AA	AB	AA	AA	F5	B
	2035	6	AA	AA	AA	AA	AA	AB	AA	AA	AA	BB	F5	A
	2029	5	AB	AA	AA	AA	AB	—	—	—	—	—	F5	B
	2036	6	AA	AA	AA	AA	AA	BB	AA	AB	AA	AA	F5	B
	2031	5	AB	AA	AA	AA	AA	AA	AA	BB	AA	AB	F5	B
	2033	5	AA	AB	AA	AA	AA	AB	AA	AA	AA	AA	F5	A + B
	2040	6	AA	AA	AA	AB	AB	AA	AA	BB	AA	AB	F5	B
	2037	5	AB	AA	AA	AA	AA	AA	AA	AB	AA	AB	F5	B
	2038	5	AA	AB	AA	AA	AA	AA	AA	AB	AA	AB	—	—
	2032	3	BB	AB	AA	AA	AA	BB	AA	AA	AA	AB	F5	B
5	2004	6	AA	AA	AA	AA	AA	AB	AA	AB	AA	AA	F5	A + B
	2002	5	AB	AA	AA	AA	AA	AB	AA	AB	AA	AA	F5	A + B

TABLE 3. CONTINUED.

Loc	ID	HI	Chromosome markers					Allozyme loci					mtDNA	rDNA
			P1	P2	P6	P3	P4	sAcoh-A	Iddh-A	Ldh-B	αMan-A	Pnp-A		
	2008	6	AA	AA	AA	AA	AA	BB	AA	AA	AA	AB	F5	A + B
	2009	6	AA	AA	AA	AA	AA	AA	AA	AB	AA	AB	F5	A + B
	2011	5	AB	AA	AA	AA	AA	AA	AA	AB	AA	AB	F5	B
	2006	4	AA	AA	AB	AB	BB	BB	AA	AA	AA	AA	F5	B
	2005	3*	AB	AB	AB	AB	AA	BB	AA	AA	AA	AB	F5	B
	2012	3*	AB	AB	AB	AA	AA	AB	AA	AB	AA	AB	F5	B
	2014	3	BB	AA	AB	AA	BB	AA	AA	AA	AA	AA	F5	B
	2013	3	BB	AA	AB	AA	AB	AA	AA	AB	AA	AA	F5	A + B
	2015	2	AA	BB	BB	AA	AA	BB	AA	BB	AA	AB	FM2	B
	2003	1	BB	BB	AB	AA	AB	AA	AA	AA	AA	AA	F5	B
	2027	0	BB	BB	BB	BB	AA	AA	AA	AA	AA	AA	FM2	B
	2028	5	AA	AA	AB	BB	AB	AA	AA	AB	AA	AB	FM2	B
6	1940	1	AB	BB	BB	BB	AB	BB	AA	AA	AA	—	FM2	B
	1962	1	BB	AB	BB	BB	BB	BB	AA	AA	AA	AA	—	—
	1964	1	AB	BB	BB	BB	AB	AB	AA	AA	AA	AB	FM2	B
	1938	0	BB	BB	BB	BB	AB	BB	AA	AA	AA	AA	—	—
	1939	0	BB	BB	BB	BB	AB	BB	AA	AA	AA	AA	—	—
	1963	0	BB	BB	BB	BB	BB	BB	AA	AA	AA	BB	—	—
	1971	0	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	—	—
	1968	0	BB	BB	BB	BB	AA	AB	AA	AA	AA	—	—	—
	1967	0	BB	BB	BB	BB	AB	AA	AA	AA	AA	AA	—	—
	1966	0	BB	BB	BB	BB	AB	AB	AA	AA	AA	BB	FM2	B
	1969	0	BB	BB	BB	BB	BB	AB	—	AA	AB	AB	—	—
	1970	0	BB	BB	BB	BB	BB	AB	—	AB	AB	AA	—	—
7, 8	1836	0	BB	BB	BB	BB	AB	AB	AA	AA	AA	AB	—	—
	1890	0	BB	BB	BB	BB	BB	AB	AA	AA	AA	AA	—	—
	1891	0	BB	BB	BB	BB	BB	AB	AA	AA	AA	AA	—	—
	1892	0	BB	BB	BB	BB	AB	AA	AA	AA	BB	AA	—	—
	1893	0	BB	BB	BB	BB	AB	AB	AA	AA	BB	AA	—	—
	1904	0	BB	BB	BB	BB	AB	AB	AA	AA	BB	AA	—	—
	1894	0	BB	BB	BB	BB	AA	AA	AA	AA	BB	AA	—	—
	1896	0	BB	BB	BB	BB	AB	BB	AA	AA	AB	AB	—	—
	1897	0	BB	BB	BB	BB	BB	AB	AA	AA	BB	AA	—	—
	1900	0	BB	BB	BB	BB	AA	AB	AA	AA	BB	AB	—	—

TABLE 3. CONTINUED.

Loc	ID	HI	Chromosome markers					Allozyme loci					mtDNA	rDNA
			P1	P2	P6	P3	P4	sAcoh-A	Iddh-A	Ldh-B	αMan-A	Pnp-A		
	1901	0	BB	BB	BB	BB	BB	AA	—	AA	AB	AB	—	—
	1898	0	BB	BB	BB	BB	AB	AB	AA	AA	BB	AA	—	—
	1849	0	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	—	—
	1905	0	BB	BB	BB	BB	AB	AB	AA	AA	BB	AB	—	—
	1902	0	BB	BB	BB	BB	AB	AB	AA	AA	AB	AA	FM2	B
	1850	0	BB	BB	BB	BB	AB	BB	AA	AA	AA	AA	—	—
	1906	0	BB	BB	BB	BB	AB	AB	—	AA	AB	AA	—	—
	1895	—	—	—	—	—	—	AA	—	AA	AB	AA	—	—

and Davis, 1989), we made a concerted effort to screen a large sample of enzyme loci and restriction sites for both the F5 and FM2 samples. Table 2 summarizes the buffer/tissue combinations used to successfully resolve 42 presumptive gene products across a subsample of 117 lizards. A total of 12 hexanucleotide restriction endonucleases was used to cut samples of genomic DNA, from a subsample of 52 lizards for Southern blotting and sequential filter hybridization with the mtDNA and rDNA probes used by Sites and Davis (1989). The 12 enzymes that consistently cut DNA of all samples were Bam HI, Bcl I, Bgl II, Eco RI, Eco RV, Kpn I, Nco I, Pst I, Pvu II, Stu I, Xba I, and Xmn I. Details of the molecular protocols are given in Sites and Davis (1989) and Arévalo et al. (1993).

Data analysis.—A subset of the allozyme data (the polymorphic loci) was analyzed by single locus genotype for all localities (for five polymorphic loci, see below) using the BIOSYS-1 program (PC version, release 1.7) of Swofford and Selander (1981). Samples were tested for conformance to Hardy-Weinberg (HW) genotypic ratios, using Levene's (1949) correction factor for small samples, and then pooled into various combinations to facilitate comparisons with mtDNA haplotype frequency data. A matrix of Nei's genetic distances (1978) was generated for pairwise comparisons by locality and then clustered with the UPGMA option to produce a phenogram representing levels of divergence across the transect. MtDNA restriction site data were analyzed with several options of version 4.0 of the REAP program (Restriction Enzyme Analysis Package, McElroy et al., 1991). Nucleotide sequence divergence was estimated from mapped site data for pairwise combinations of haplotypes (the d_r values of Nei and Tajima, 1981; Nei and Miller, 1990), and standard errors were estimated for all d_r values (Nei, 1987; Nei and Tajima, 1983). MtDNA haplotypes were also analyzed in a locality × locality comparison, using a weighted version of the same d_r values to produce a matrix (additional details are given in Arévalo et al., 1993), which was depicted as a phenogram generated by UPGMA. We also used the Monte Carlo simulation test for geographic heterogeneity in haplotype frequencies (through a χ^2 analysis) in the manner described by Arévalo et al. (1993; details given by Roff and Bentzen, 1989). We quantified single-copy nuclear allele frequency heterogeneity by calculating F_{st} values for the b alleles at each of the five polyallelic loci, using the θ estimator described by Weir and Cock-

erham (1984). Estimates were obtained with computer programs described by Weir (1990, chapter 5), and employed a jackknife procedure over all populations (n = 6) and then over all loci (n = 5) to estimate variances for θ (presented as standard deviations).

Results

Single-copy nuclear (scn) markers: chromosomes.— Conventionally stained mitotic karyotypes should show five fixed differences between the F5 and FM2 cytotypes. However, even in the best preparations, pairs 3 and 4 are difficult to distinguish in the absence of meiotic pairing data, and microchromosomes are difficult to resolve with certainty in all but exceptional preparations. Chromosome pairs 1, 2, and 6 provided unambiguous fixed differences between these two races, segregating as unlinked single-copy Mendelian loci and were, therefore, useful for characterizing this zone.

Figure 2 illustrates representative mitotic karyotypes from lizards sampled from the Tulancingo transect. Panel A shows a typical F5 female in which all macrochromosomal pairs (except 5) retain the presumed ancestral biarmed morphology, and the ancestral number of microchromosomes (10 pairs; 2n = 34). Panel B shows an individual homozygous for a pericentric inversion at pair 4, whereas the karyotypes depicted in panels C, D, and E represent different combinations of hybridization with F5 individuals (heterozygous for one or two diagnostic markers in an otherwise largely biarmed karyotype), and those in panels F and G likely represent additional combinations of F_2 generation animals. Figure 2H shows an anomalous individual that is trisomic at pairs 4 and 6. Note that, strictly on the basis of chromosomal markers at pairs 1, 2, and 6, the individuals depicted in panels C and E would be classified as F5, even though they carry acrocentrics at pairs 3 and/or 4. We highlight these observations simply to alert readers to the limitations of our scoring protocols; some uncertainties are unavoidable in the absence of more detailed analyses.

On the right side of Figure 2, panel P depicts a typical FM2 female with an entirely acrocentric macrochromosomal complement, plus 11 pairs of microchromosomes (2n = 46). On the basis of the three unambiguous markers used to define the hybrid zone, the karyotypes depicted in panels I and J would both be classified as F_1 hybrids (heterozygous for the diagnostic rearrangements at pairs 1, 2, and 6). Based on the number of metacentric elements at pairs 3 and 4, the individual depicted in panel I is likely closer to a pure F_1 hybrid, whereas the individual in panel J may be introgressed by F5 chromosomes at pair 3 and/or 4. Individuals depicted in panels K–N are either first generation backcrosses to FM2 (heterozygous for diagnostic rearrangements at one or two of the markers, but not all three, in an otherwise largely acrocentric karyotype), or later generation backcross individuals in the same direction (toward FM2; as in Fig. 2, panel O).

Chromosomal morphologies were coded into genotypes following the previously established protocols of Porter and Sites (1986) and Arévalo et al. (1991). Biarmed chromosomes were designated individually by an "A," the alternative pair of acrocentric fission products as "B," and the pair 4 pericentric inversion as "C." Table 3 summarizes the distribution of chromosomal genotypes at all sampling points along the Tulancingo transect (samples 2 and 3 were combined, as were samples 7 and 8, due to small sample sizes, geographic proximity, and chromosomal uniformity); and Figure 3 plots the frequency shift of AA genotypes for pairs 1–4 and 6 as a function of the distance west of Tulancingo. The transition is very sharp and strongly concordant for the three diagnostic markers: there is a virtually complete transition from the F5 to the FM2 karyotype at pairs 1, 2, and 6 over a geographic distance of 2–3 km. The transitions evident in pairs 3 and 4 must be interpreted cautiously because unambiguous scoring of these pairs is confounded by their morphological similarity and the fission polymorphism at pair 4 within the FM2 race (Porter and Sites, 1986; Arévalo et al., 1991). The tabulation of genotypes for these pairs in Table 3, although verified by meiotic pairing behavior in adult males, is also tentative for some individuals.

To visualize the transition in terms of the proportion of individual genotypes at each locality, a hybrid index was calculated for all lizards scored for the three diagnostic markers. Each metacentric chromosome at pairs 1, 2, or 6 was given a numerical score of 1, so that a pure F5 (with six metacentrics) would be scored as 6, and a pure FM2 as 0. F_1 and backcross individuals would fall somewhere in between, depending upon the total number of metacentrics at the three markers. Figure 4 depicts the distribution of these hybrid scores at all sites along the transects, and individual hybrid index scores (HI) are given for all chromosomal genotypes summarized in Table 3. Localities 1 and 7–8 are comprised of chromosomally pure F5 and FM2 individuals, respectively; however modest introgression of FM2 chromosomes is

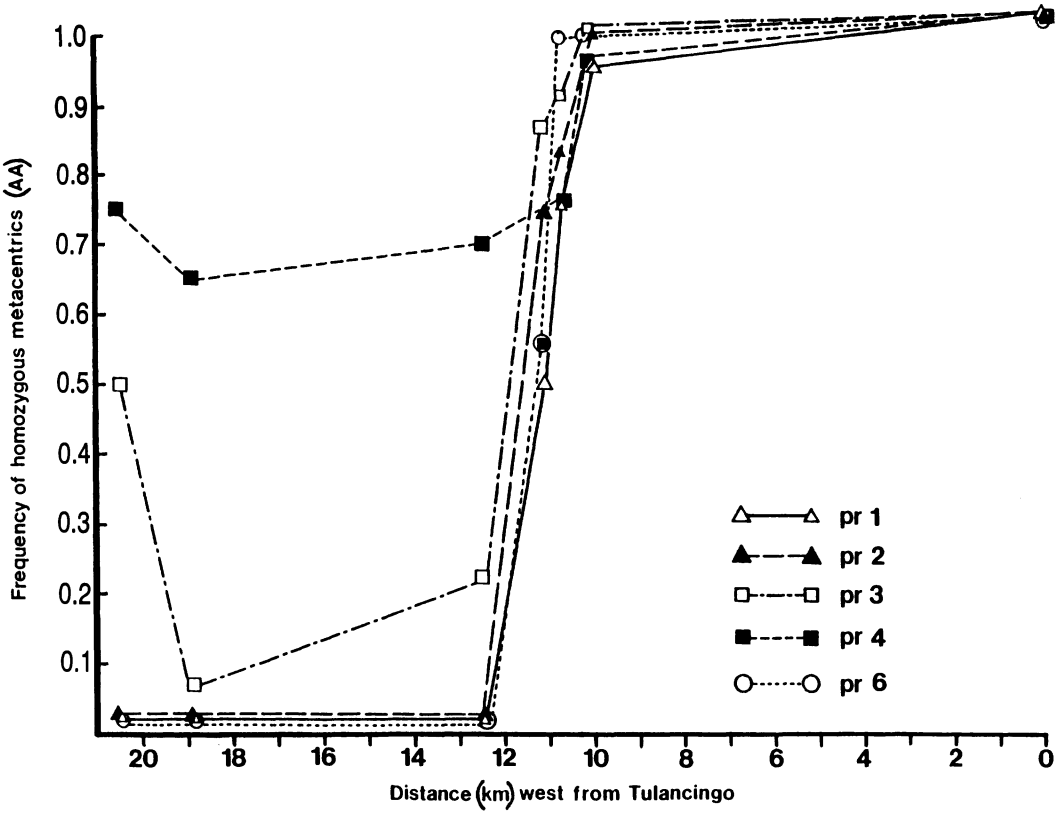

Fig. 3. Distribution of single macrochromosome pair genotypes among populations of *Sceloporus grammicus* sampled from the Tulancingo transect along Hwy 130 (see Fig. 1).

apparent at locality 2–3, and F5 markers appear in low frequency at locality 6. In both cases, this introgression is due to the flow of single markers in a small number of individuals at each of these localities (Table 3). Localities 4 and 5 contained different classes of individuals of "mixed parentage." The majority of animals at site 4 are some combination of backcross-to-F5 genotypes, whereas the sample from locality 5 includes two F_1 hybrids (Fig. 2I, J; Table 3), and both B × F5 and B × FM2 genotypes.

Scn markers: allozymes.—The screening of 42 presumptive gene loci (Table 2) failed to identify any fixed allozyme differences between the F5 and FM2 cytotypes. In fact, divergence is surprisingly low between these populations in view of their pronounced chromosomal differences and given the modest levels of divergence between some other cytotypes (Hall and Selander, 1973; Sites et al., 1988). Twenty-six of the 42 loci surveyed (61%) were fixed for the same allozymes across all individuals examined, including Adh-A, both AAT loci, Ck-A, both FBP loci, Fum-A, βGa, βGala-2, Gp-1, Gpi-A, βGlur-A, Glal-A, both GR loci, mMdh-A, Mpi-A, all PEP loci, Pgdh-A, all SOD loci, and Tpi-A. Ten additional loci (sAcoh-A, Ddh-A, βGala-1, αGlus-A, Icdh-1, Ldh-A, sMdh-A, Mdhp-1, Pgm-A, and Pgk-A) displayed very low levels of polymorphism in the form of hetero- or homozygotes in single individuals at one or two localities. The locus G3pdh-A has been duplicated in other populations of the *Sceloporus grammicus* complex (Sites and Murphy, 1991), and the large number of three-banded isozyme phenotypes for this locus (21 of 22) from locality 1 suggests the presence of a duplication in this population. This may still serve as a useful marker, but we do not consider it further in this paper until additional data become available to clarify its genetic basis in the F5 cytotype.

The five remaining loci all segregated two alleles in some samples across the Tulancingo transect, and their genotypic distributions are summarized in Table 3 and plotted in Figure 5. One locus, sAcoh-A, segregates for a second (b) allele in the western part of the transect, but this allele is rare in the eastern sites. Two loci, Iddh-A and Ldh-B, show the reverse pattern,

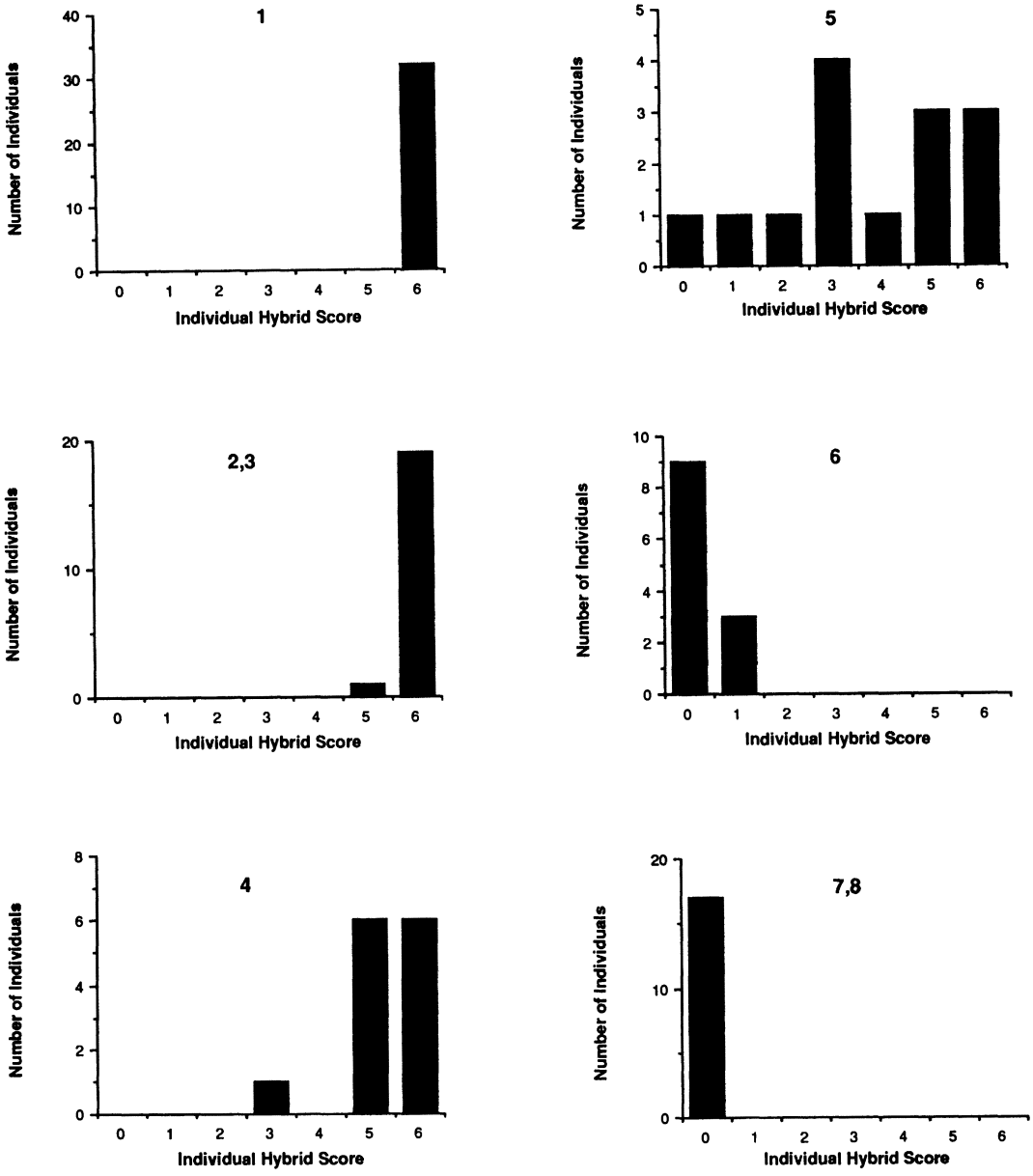

Fig. 4. Distribution of individual chromosomal hybrid index scores among populations of *Sceloporus grammicus* sampled from east (locality 1) to west (localities 7 and 8) across the Tulancingo transect. Hybrid scores denote the number of F5 metacentric chromosomes at the three diagnostic markers, and range from 0 (all acrocentrics) to 6 (all metacentrics at pairs 1, 2, and 6).

segregating for alternative alleles in the F5 samples in the easternmost part of the transect but with one allele being rare or absent in the western samples. The fourth locus, Pnp-A, segregated for a second allele (b) in decreasing frequencies from east to west. The fifth marker, αMan-A, was unusual in that it segregated for a second allele (b) at the easternmost and westernmost ends of the transect but was monomorphic for the (a) allele at three intermediate localities (sites 2,3, 4, and 5; Table 3).

These five loci were used to statistically characterize population structure and allozyme divergence among samples across the Tulancingo transect. In addition to the combined localities mentioned above, we also added animals from site 6 to the 7,8 sample, primarily to produce a data matrix amenable to direct comparison to

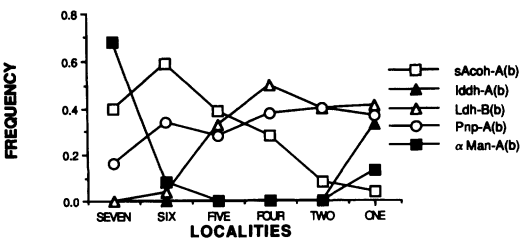

Fig. 5. Distribution of (b) allele frequencies for five isozyme loci (Table 3) in samples of *Sceloporus grammicus* across the Tulancingo transect; numbers on the horizontal axis refer to the sampling localities in Table 1 and Figure 1.

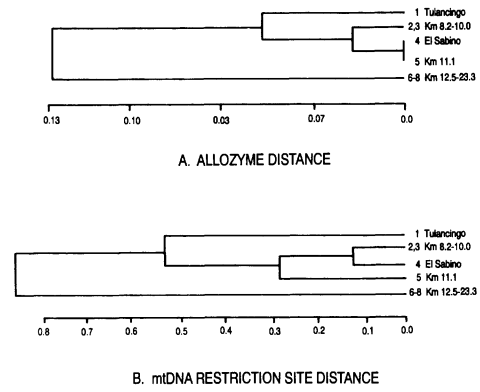

Fig. 6. UPGMA phenograms of (A) Nei's (1978) genetic distances based upon allele frequencies at five isozyme loci (Table 4, below diagonal), and (B) Nei and Tajima's (1981) mtDNA restriction site-based distances (Table 4, above diagonal) for all pairwise comparisons of localities for *Sceloporus grammicus* across the Tulancingo transect.

the mtDNA matrix (for which the same combinations had to be used to augment sample sizes; see below). All of the samples that were now considered separate OTUs for further comparisons (1, 2 + 3, 4, 5, and 6–8) were tested for conformance to expected HW genotypic ratios, and all fit with two exceptions. For the OTU designated 6–8, three of four polymorphic loci had genotypic ratios conforming to random mating expectations, whereas αMan-A showed a statistically significant deficiency of heterozygotes (χ^2 with 1 df = 4.806, $P < 0.028$), but this undoubtedly reflects a Wahlund effect resulting from combining sample 6, where the (a) allele is in highest frequency, with sample 7 + 8, in which this is the uncommon allele (Table 3). The only other deviation from HW genotypic ratios was seen in the sAcoh-A locus at locality 5 (χ^2 with 1 df = 4.882, $P < 0.027$), but this sample contained a substantial number of animals with mixed parentage (Fig. 4), and the heterozygote deficiency could have a number of explanations.

Table 4 presents pairwise comparisons for five OTUs, of Nei's (1978) genetic distance coefficients (below diagonal) calculated over the five variable loci plotted in Figure 5. Nei's D values ranged from a low of 0.001 to a high of 0.174, with the highest values consistently being those for OTU 6–8 (Table 4). These relationships are depicted as a UPGMA phenogram in Figure 6A. As expected from the matrix, sample 6–8, consisting mostly of FM2 animals, was the most distinct, and a second smaller dichotomy separated sample 1 (the chromosomally pure F5 cytotype) from all localities in the middle region of the transect (OTUs 2–3, 4, and 5). Levels of divergence between all combinations of these midtransect OTUs were relatively low (Table 4; Fig. 6A).

TABLE 4. MATRIX OF ALLOZYME GENETIC DISTANCES BASED UPON FIVE POLYMORPHIC LOCI (Nei, 1978; below diagonal) AND WEIGHTED mtDNA DISTANCES (Nei and Tajima, 1981; above diagonal) FOR ALL PAIRWISE COMBINATIONS OF *Sceloporus grammicus* SAMPLED ALONG THE TULANCINGO TRANSECT.

Locality	1	2, 3	4	5	6–8
1	—	0.056	0.050	0.057	0.082
2, 3	0.029	—	0.013	0.032	0.094
4	0.049	0.001	—	0.024	0.086
5	0.065	0.024	0.001	—	0.067
6–8	0.174	0.151	0.131	0.171	—

For each locus, we present results of these tests in the form of the θ value, followed in parentheses by the mean and standard deviation of the jackknife-across-population resampling option. In alphabetical order, these are sAcoh-A(b) − 0.2996 (X = 0.3089 + 0.1165); Iddh-A(b) − 0.2977 (X = 0.2599 + 0.1633); Ldh-B(b) − 0.1645 (X = 0.1356 + 0.1202); αMan-A(b) − 0.0076 (X = 0.0126 + 0.0230); and Pnp-A(b) − 0.0696 (X = 0.0627 + 0.0577). These values indicate pronounced heterogeneity in θ values between loci, and the θ value for all loci is 0.1933 (X = 0.1942 + 0.0563). Some of our sample sizes were too small to permit proper use of a χ^2 statistic to test the null hypothesis that θ differed significantly from 0.0 (complete panmixia), but a θ value of 0.1933 over all loci reflects substantial heterogeneity in allele frequencies between sampling localities across the Tulancingo transect.

Multicopy nuclear markers: rDNA genotypes.—A total of 52 animals was surveyed for rDNA re-

TABLE 5. DISTRIBUTION OF *Sceloporus grammicus* mtDNA HAPLOTYPES ACROSS THE TULANCINGO TRANSECT. Samples (Loc) refer to localities in Table 1 and Figure 1; ID = museum catalog (CAP) number of each genotype, the two F_1 hybrid animals from locality 5 are indicated by "*"; mtDNA haplotype refers to fragment patterns identified from each restriction endonuclease, in the following order: Bam HI, Bcl I, Kpm I, Pvu II, Pst I, Nco I, Eco RV, Xba I, Xmn I, Stu I, and Bgl II; haplotype codes are those cross-listed in Table 3. The rDNA types are based on a single site difference at the enzyme Xmn I.

Loc	ID	Haplotypes											Class	Race	rDNA
1	1599	A	A	A	A	A	A	A	A	A	A	A	I	F5	A
	1602	A	A	A	A	A	A	A	A	A	A	A	I	F5	A
	1603	C	B	B	B	A	B	B	B	B	B	B	VII	F5*	A
	1604	A	A	A	A	A	A	A	A	A	A	A	I	F5	A + B
	1605	C	B	B	B	A	B	B	B	B	B	B	VII	F5*	A
	1606	C	B	B	B	A	B	B	B	B	B	B	VII	F5*	A
	1607	A	A	A	A	A	A	A	A	A	A	A	I	F5	A
	1817	C	B	B	B	A	B	B	B	B	B	B	VII	F5*	A
	1818	A	A	A	A	A	A	A	A	A	A	A	I	F5	A
	1824	C	B	B	B	A	B	B	B	B	B	B	I	F5	A + B
2, 3	1958	A	A	A	A	A	A	A	A	A	A	A	I	F5	B
	1959	A	E	A	A	A	D	C	A	D	D	A	VI	F5	B
	1960	A	A	A	D	A	A	A	A	A	A	A	III	F5	A + B
	2043	A	A	A	A	A	A	A	A	A	E	A	IV	F5	—
	2046	A	A	A	A	A	A	A	A	A	E	A	IV	F5	B
	2048	A	E	A	A	A	D	C	A	D	D	A	VI	F5	A + B
	2049	A	E	A	A	A	D	C	A	D	D	A	VI	F5	A + B
	2050	A	E	A	A	A	D	C	A	D	D	A	VI	F5	A + B
	2051	A	E	A	A	A	D	C	A	D	D	A	VI	F5	B
	2053	A	E	A	A	A	D	C	A	D	D	A	VI	F5	A
	2054	A	E	A	A	A	D	C	A	D	D	A	VI	F5	A + B
	2059	A	E	A	A	A	D	C	A	D	D	A	VI	F5	B
	2056	A	E	A	A	A	D	C	A	D	D	A	VI	F5	B
	2057	A	E	A	A	A	D	C	A	D	D	A	VI	F5	B
	2060	A	E	A	A	A	D	C	A	D	D	A	VI	F5	B
4	2035	A	A	A	A	A	A	A	A	A	A	A	I	F5	A
	2036	A	A	A	A	A	A	A	A	A	E	A	IV	F5	B
	2037	A	E	A	A	A	D	C	A	D	D	A	VI	F5	B
	2040	A	A	A	D	A	A	A	A	A	A	A	III	F5	B
	2041	A	A	A	A	A	A	A	A	A	A	A	I	F5	B
	2029	A	A	A	A	A	A	A	A	A	A	A	I	F5	B
	2030	A	E	A	A	A	D	C	A	D	D	A	VI	F5	B

TABLE 5. CONTINUED.

Loc	ID	Haplotypes											Class	Race	rDNA
	2031	A	A	A	A	A	A	A	A	A	E	A	IV	F5	B
	2032	A	A	A	A	A	A	A	A	A	A	A	I	F5	B
	2033	A	A	A	A	A	A	A	A	A	A	A	I	F5	A + B
5	2002	A	A	A	A	A	A	A	A	A	A	A	I	F5	A + B
	2003	A	A	A	A	A	A	A	A	A	A	A	I	F5	B
	2004	A	A	A	A	A	A	A	A	A	A	A	I	F5	A + B
	2005*	A	A	A	A	A	A	A	A	A	A	A	I	F5	B
	2006	A	D	A	A	A	A	A	D	D	A	A	V	F5	B
	2008	A	E	A	A	A	D	C	A	D	D	A	VI	F5	A + B
	2009	A	A	A	A	A	A	A	A	A	A	A	I	F5	A + B
	2011	A	A	A	A	A	A	A	D	A	A	A	II	F5	B
	2012*	A	A	A	A	A	A	A	A	A	A	A	I	F5	B
	2013	A	A	A	A	A	A	A	A	A	A	A	I	F5	A + B
	2014	A	A	A	A	A	A	A	A	A	A	A	I	F5	B
	2015	B	C	A	C	B	C	D	C	C	C	B	IX	FM2	B
	2027	B	C	A	C	B	C	D	C	C	C	B	IX	FM2	B
	2028	B	C	A	C	B	C	D	C	C	C	B	IX	FM2	B
6	1940	B	C	A	C	B	C	D	C	C	C	B	IX	FM2	B
	1963	B	C	A	C	B	C	D	C	C	C	B	IX	FM2	B
	1964	B	C	A	C	B	C	C	C	C	C	B	VIII	FM2	B
7, 8	1902	B	C	A	C	B	C	C	C	C	C	B	VIII	FM2	B

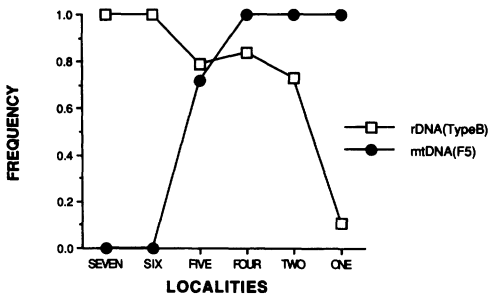

Fig. 7. Distribution of type-B rDNA repeat and F5 mtDNA haplotypes (Tables 3, 5) in samples of *Sceloporus grammicus* across the Tulancingo transect; numbers on the horizontal axis are as in Figure 5.

striction site variation with 11 restriction endonucleases. All animals were identical in fragment pattern for 10 of these enzymes, including Bam HI, Bcl I, Dra I, Eco RI, Eco RV, Nco I, Pst I, Pvu II, Stu I, and Xba I. A single restriction site difference was found at the enzyme Xmn I, and the two rDNA repeat types were designated as A and B. The distribution of both types, as well as a third "A + B" type, is summarized in Tables 3 and 5, and Figure 7 displays the frequency shift of the type-B rDNA repeat across the Tulancingo transect. The two types did not exhibit fixed differences between the F5 and FM2 cytotypes but did show a frequency shift across the zone. From east to west, the shift in the frequency of type B is as follows: 1, 0.11; 2,3, 0.73; 4, 0.84; 5, 0.80; 6, 1.0; and 7,8, 1.0. Note that there is a pronounced frequency change in rDNA types between localities 1 and 2,3, several kilometers east of the sharp transition between the three chromosomal markers that define the F5 × FM2 contact (Fig. 3). This skewed rDNA transition suggests that the transect in this study was inadequate to cover the complete geographic transition in all markers and that samples taken from further east (east of the town of Tulancingo) would have revealed that populations were fixed for rDNA repeat type A. The extremely low level of rDNA divergence between these two races contrasts markedly with estimates between the HS, LS, and F6 races, which show much greater divergence in rDNA repeats despite minimum or no divergence of their karyotypes (Arevalo et al., 1993).

Cytoplasmic markers: mtDNA haplotypes.—The same 52 animals used in the rDNA survey were screened for mtDNA haplotype diversity with 12 restriction endonucleases (the same 11 used in the rDNA screening, plus Bgl II). This analysis revealed high levels of mtDNA polymorphism across the Tulancingo transect because only a single enzyme (Eco R1) was invariant. A total of nine different mtDNA haplotypes were identified based on restriction fragment patterns, and the two cytotypes were characterized by particular groups of haplotypes. Each haplotype was defined by a composite set of 11 fragment patterns, one for each of the 11 enzymes that revealed restriction site variation between two or more animals (details of the nature of these sites are given in Sites and Davis, 1989, for FM2). Each animal is identified by a code of 11 letters following the method described by Arévalo et al. (1993). Each is also designated by a shorter code (e.g., F5 type-I) which identifies all individuals in Table 3; the complete haplotypes are given in Table 5.

Because the restriction sites responsible for the fragment patterns were not mapped in the F5 cytotype in the earlier study by Sites and Davis (1989; F5 was poorly known when that study was carried out), we inferred site differences between fragment patterns on the basis of number and size of fragments for each pattern. By checking this approach with similar inferences made from FM2 fragment patterns, but having known site maps for the FM2 race, we discovered that site differences among F5 individuals could be reliably inferred for all enzymes except Stu I. Following the approach taken by Arévalo et al. (1993), we used 10 enzymes to define eight haplotypes [all but IV (AAAAAAAAAEA) in Table 5] for subsequent statistical analyses. Table 6 summarizes estimates of mtDNA sequence divergence and their standard errors among all pairwise combinations of this subset of eight haplotypes.

Distance estimates ranged from a low value of 0.003 between two of the FM2 haplotypes (class VIII and class IX) which differed by a single XbaI site, to a high value of 0.106 between two of the F5 haplotypes (class V and class VII). The class V haplotype was present in a single individual at locality 4 and represents a minimum level of divergence from class VII haplotypes of 23 sites. Further extensive subdivision was apparent within the F5 cytotype at locality one (city of Tulancingo), in which the class I and class VII haplotypes differed by a minimum of 21 sites (d_r value = 0.095, see Table 6). This result was unexpected given the location of this sample well outside of the hybrid zone (Fig. 1).

Table 4 presents pairwise comparisons of weighted haplotype d_r values (mtDNA distances of Nei and Tajima, 1981; above diagonal) for the same OTUs defined above for the allozyme

TABLE 6. ESTIMATES OF EVOLUTIONARY DISTANCE 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 THE EIGHT HAPLOTYPES (TYPES I AND IV ARE COMBINED DUE TO A Stu I SITE UNCERTAINTY) OF *Sceloporus grammicus* IDENTIFIED FROM THE TULANCINGO TRANSECT WITH 10 ENZYMES.

Haplotype	I	VII	VI	III	V	II	IX	VIII
I	—	0.027	0.009	0.004	0.006	0.004	0.024	0.022
VII	0.095	—	0.027	0.027	0.028	0.027	0.024	0.022
VI	0.019	0.099	—	0.010	0.007	0.010	0.028	0.025
III	0.004	0.099	0.022	—	0.007	0.005	0.025	0.023
V	0.011	0.106	0.014	0.014	—	0.005	0.026	0.024
II	0.004	0.099	0.022	0.007	0.007	—	0.025	0.023
IX	0.086	0.086	0.102	0.090	0.097	0.090	—	0.003
VIII	0.078	0.078	0.093	0.082	0.088	0.082	0.003	—

comparisons. Estimates of mtDNA between-sample divergence ranged from a low value of 0.013 to a high of 0.094. The composite OTU 6–8, representing mostly FM2 animals, was consistently the most distinct in its mtDNA profile; and its position is reflected in the UPGMA phenogram derived from the d_r matrix (Fig. 6B). The branching topology of the mtDNA tree is similar to the allozyme-based tree (Fig. 6A) in that the secondmost distinctive OTU is locality 1 (the F5 cytotype); and the midtransect localities form a third well-defined group.

Figure 7 summarizes the frequency shift in F5 haplotypes (all classes taken together as a single marker) across the Tulancingo transect. Mitochondrial DNA introgression across the zone appeared to be limited (within the constraints of our small sample sizes) to intermediate localities (4 and 5). All individuals with parental karyotypes had their respective mtDNA genomes. All individuals with hybrid index scores of 1 or 2 (genotypes diagnostic of backcross-to-F5 or F_2 individuals) at the intermediate localities had F5 mtDNAs, and four of five individuals with hybrid index scores of 4 or 5 (backcross-to-FM2 or F_2 genotypes) had FM2 mtDNAs. However, five individuals had hybrid index scores of 3, indicating either F_1 hybrids of offspring of two hybrid parents, and all of these had F5 mtDNAs (Table 3).

We analyzed the geographic heterogeneity of mtDNA haplotype frequencies using the Monte Carlo simulation approach described by Roff and Bentzen (1989). Table 7 summarizes the distribution of haplotypes by locality across the Tulancingo transect, and the χ^2 value calculated from this matrix was 90.7. The distribution of χ^2 values from 1000 simulations averaged 28.4, with minimum and maximum values of 15.1 and 56.1, respectively. Because none of the replicate χ^2 values exceeded that of the original matrix, the probability of attaining a value higher than the original χ^2 value by chance alone is 0, indicating highly significant heterogeneity in the distribution of mtDNA haplotypes.

Morphological divergence.—The same meristic characters were recorded for all animals karyotyped from the Tulancingo transect as in the companion study, and these were collected by the same investigator (G. Lara). The 10 variables were analyzed by several subroutines of Statistical Analysis Systems, in the same manner as described by Arévalo et al. (1993). A stepwise discriminant analysis (SDA) was used to identify characters having the highest power of discrimination between the two parental cytotypes. The SDA analysis revealed six characters that maximized separation of the F5 and FM2 cytotypes: dorsal spots (DS), frontoparietials (FP), infralabials (IL), internal labiomentals (INL), subdigital lamellae (SDL), and supraoculars (SO). These six characters were used in a canonical discriminant analysis (CDA) to ordinate all individuals assigned a priori to one of three genotypic classes on the basis of the three diag-

TABLE 7. DISTRIBUTION OF EIGHT mtDNA HAPLOTYPES OF *Sceloporus grammicus* IDENTIFIED BY 10 ENZYMES AMONG ALL SAMPLES SURVEYED FROM THE TULANCINGO TRANSECT. Locality numbers refer to collecting sites depicted in Figure 1, and roman numerals identify mtDNA classes summarized in Table 5.

Locality	I	VII	VI	III	V	II	IX	VIII
1	6	4	0	0	0	0	0	0
2, 3	3	0	11	1	0	0	0	0
4	7	0	2	1	0	0	0	0
5	8	0	1	0	1	1	3	0
6–8	0	0	0	0	0	0	2	2

TABLE 8. SUMMARY OF CHARACTERS IDENTIFIED BY A STEPWISE DISCRIMINANT ANALYSIS TO SHOW MAXIMUM DIVERGENCE BETWEEN THE F5 AND FM2 CYTOTYPES OF *Sceloporus grammicus* ACROSS THE TULANCINGO TRANSECT. Localities are those in Table 1 and Figure 1; and the data summarized include the mean count and ± standard error per sample, and the range below in parentheses.

Locality	n	Dorsal spots	Frontoparietals	Infralabials	Internal labiomentals	Subdigital lamellae	Supraoculars
1	32	7.64 ± 1.0 (5–10)	3.45 ± 1.3 (2–8)	13.30 ± 1.0 (10–16)	6.30 ± 2.1 (3–11)	45.15 ± 2.0 (41–51)	12.55 ± 1.3 (10–17)
2, 3	20	7.36 ± 1.5 (4–11)	2.57 ± 0.2 (2–5)	12.04 ± 1.3 (10–15)	8.57 ± 2.5 (3–14)	42.43 ± 2.7 (40–49)	13.14 ± 3.1 (8–16)
4	14	7.46 ± 1.0 (6–9)	2.92 ± 0.1 (2–4)	11.77 ± 1.0 (10–14)	9.54 ± 2.2 (5–13)	44.00 ± 3.2 (39–50)	12.46 ± 1.8 (10–16)
5	14	7.87 ± 1.0 (6–10)	3.20 ± 0.1 (2–4)	12.20 ± 1.0 (10–14)	9.53 ± 3.2 (4–14)	42.40 ± 2.6 (39–48)	13.60 ± 1.5 (10–16)
6	12	9.14 ± 1.4 (7–11)	2.71 ± 0.5 (2–4)	11.86 ± 0.5 (11–13)	8.07 ± 3.1 (4–16)	40.14 ± 1.8 (38–44)	13.07 ± 1.2 (11–14)
7, 8	18	10.59 ± 1.4 (8–13)	2.56 ± 0.2 (2–4)	12.81 ± 2.4 (10–15)	6.63 ± 1.7 (2–10)	40.34 ± 2.2 (36–46)	13.88 ± 1.1 (12–16)

nostic chromosome markers. All lizards with a hybrid index score of 6 were classified as pure F5, those with scores of 0 were considered pure FM2, and all animals with scores ranging from 1 to 5 were designated as "hybrids."

Table 8 summarizes the means, standard deviations, and ranges for these six parameters (numbers are totals on both sides of the body) at all localities sampled across the Tulancingo transect. The frequency shifts are slight, but four characters do show clinal shifts from the F5 to the FM2 cytotypes. For example, the total number of dorsal spots (DS) increases from east ($\bar{X}$ = 7.6 at site 1) to west ($\bar{X}$ = 10.6 at sites 7,8), whereas both numbers of frontoparietals (FP) and subdigital lamellae (SDL) decrease from east to west ($\bar{X}$ = 3.4 and 2.6 at sites 1 and 7,8, respectively, for FP, and $\bar{X}$ = 45.1 and 40.3 at sites 1 and 7,8, respectively, for SDL). A slight zigzag pattern was apparent in number of supraoculars (SO), but the overall clinal shift was in the direction of an east-to-west increase ($\bar{X}$ = 12.6 and 13.9 at sites 1 and 7,8, respectively). The number of infralabials (IL) showed a pronounced zigzag pattern across the transect, and the number of internal labiomentals (INL) was unique in its bell-shaped distribution; the highest counts were recorded from the center of the hybrid zone itself ($\bar{X}$ counts of 9.54 and 9.53 at sites 4 and 5, respectively), and tapered to lower counts at either side of the transect ($\bar{X}$ counts of 6.30 and 6.63 at sites 1 and 7,8, respectively).

Figure 8 shows results of the CDA analysis as frequency distributions of individual lizards along a single canonical discriminant variable (the horizontal axis) for the three classes of chromosome genotypes just described. The CDA revealed statistically significant separation and minimum overlap between lizards scored as pure F5 and FM2 genotypes (Wilks lambda = 0.15; df = 6, 109; $P < 0.01$). All "hybrid" animals are approximately intermediate in morphology but with a distribution skewed toward the FM2 phenotype. The canonical correlation coefficient for this axis is 0.923, and correlations of the six characters with the canonical variable (FP = 0.957, DS = 0.707, SDL = −0.693, SO = 0.252, INL = −0.119, and IL = 0.008) indicate that the first four of these contribute most heavily to the separation of the F5 and FM2 cytotypes. F5 individuals generally have more dorsal spots, a higher number of frontoparietals and supraoculars, and fewer subdigital lamellae than FM2 individuals.

Results of the CDA analyses were used to estimate the probability that any given animal can be correctly assigned to its proper chromosome race on the basis of its morphological characteristics. This was carried out for animals from localities 1 (pure F5) and 7,8 (pure FM2), and a total of 16 individuals classified as pure for one race or the other (i.e., having hybrid index scores of 0 or 6), but taken from sites 2,3, 4, and 6 in the center of the transect. Fifteen of the 16 midzone animals were correctly classified into their parental races on the basis of their morphologies. This means that the likelihood of assigning an F5 or FM2 animal to its appropriate chromosome race on the basis of its morphology alone is high (probability of misclassification <0.05 in all but three cases). A single animal from site 5 (CAP 2004) with a

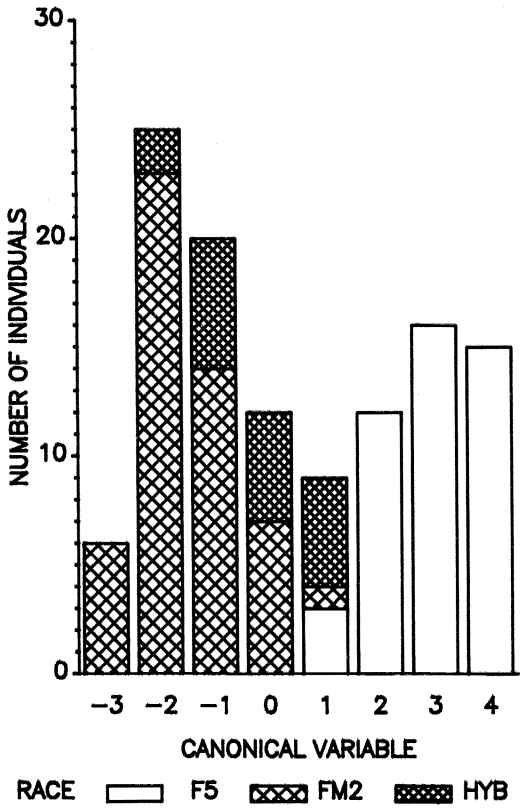

Fig. 8. Frequency distribution of morphological classes F5, FM2, and "hybrid" individuals of *Sceloporus grammicus* along a single canonical discriminant axis, which ordinates six meristic variables that maximize separation of parental types. Morphological data are included for all animals taken from the Tulancingo transect with known chromosomal genotypes.

pure F5 genotype was incorrectly assigned to the FM2 race on the basis of its morphology (posterior probability of membership in the F5 race = 0.42). Two other animals were correctly classified as F5 individuals but with low posterior probabilities (CAP 2029, locality 4, posterior probability = 0.71; and CAP 2007, locality 5, posterior probability = 0.55). Using the model obtained from the CDA, posterior probabilities of membership in each of the parental races for various "hybrid" genotypes (i.e., those with hybrid index scores of 1–5) were calculated. The probabilities of being classified with one of the parental races based on morphology increased as hybrids became more similar genotypically to one or the other parental race (Fig. 9). These results indicate that there is measurable morphological divergence between the two chromosome races and that hybrids identified karyotypically are intermediate between the parental races.

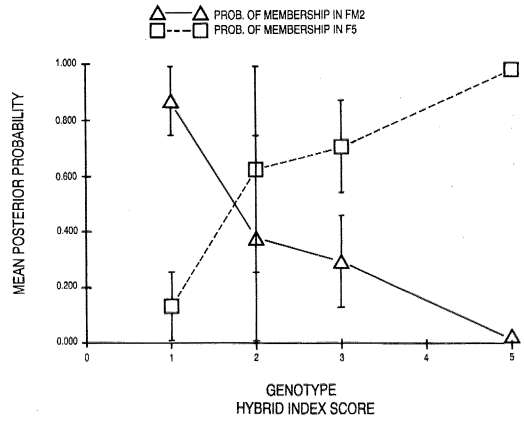

Fig. 9. Posterior probability of classification of "hybrid" individuals (identified by their hybrid index scores on the horizontal axis) into parental cytotypes (F5 or FM2), on the basis of meristic characters, for all categories of *Sceloporus grammicus* from the Tulancingo transect. Each symbol is located at the mean probability value for a particular genotype, and vertical bars give standard deviations for each mean.

Discussion

Shape of the Tulancingo hybrid zone.—A number of recent reviews of hybrid zones—narrow regions in which genetically distinct populations meet, mate, and produce hybrids—have emphasized their potential for providing information concerning the nature and effects of differences among incipient species and how selection working at different levels of the genome affects the spread and establishment of alternate adaptations (Barton and Hewitt, 1985, 1989; Harrison, 1991). Hybrid zones are known from a wide variety of plants and animals, and generally exhibit the following features. First, they are usually narrow relative to the total geographic ranges of the species (or divergent populations) involved and relative to the migration distances per generation. Second, most zones involve changes in a variety of nuclear characters, which frequently cluster in parallel gradients of frequency shifts (each gradient separately is referred to as a cline). Third, mtDNA haplotype shifts are frequently discordant with those shown by nuclear markers. Fourth, there are often strong associations between particular genotypes (linkage disequilibrium) generated by a continual diffusion of parental combinations of genes into the center of a hybrid zone and their subsequent breakdown by recombination and segregation in hybrids.

We do not have sample sizes large enough to rigorously address linkage disequilibrium issues, but several patterns evident in our data

are consistent with the first three general patterns described above. For example, from a purely geographic perspective, the width of the zone appears to be very narrow; the shift in the three diagnostic chromosomal markers shows a complete transition from AA to BB genotypes over a distance of about 2 km (between localities 4 and 6 in Fig. 1; see also Fig. 3). This apparently steep transition must be assessed, however, in the context of dispersal rates of individuals in these populations, and some estimate of the number of generations the hybridizing populations have been in contact. Neither of these two parameters is known for any of the cytotypes of the *S. grammicus* complex, but some estimates can be made from data for dispersal rates in other lizards (defined as σ, the distance moved in a generation; see Barton and Hewitt, 1989). Dispersal estimates made by Tinkle (1965) in Texas populations of the phrynosomatid *Uta stansburiana* averaged 20 m over the course of an individual's lifetime, whereas similar estimates for *Sceloporus olivaceus* averaged 78 m (Kerster, 1964). These species are smaller and larger in body size, respectively, than *S. grammicus*, and probably represent under- and overestimates of dispersal in *S. grammicus*. The majority of hybrid zones are less than 50σ wide, so substituting these dispersal estimates from other lizards, we can estimate upper (50×78 m = 3900 m) and lower limits (50×20 m = 1000 m) for what might be "narrow" in the hybrid zones of *S. grammicus*. If these estimates are close, then both the Ajusco and Tulancingo transects qualify as narrow with respect to the larger estimate, which suggests that selection limits the introgression of diagnostic markers in either direction (see Arévalo et al., 1993, for details and an apparent exception at one marker). However, the zone is wider than 50σ if the estimate for *Uta* is closer to the average dispersal per generation in these populations of *S. grammicus*, which would imply less intense selection against introgression. Mark-release-recapture studies are currently in progress at a site within the range of the F5 race east of the Tulancingo transect to obtain relevant dispersal estimates.

The second generality given above, that independent diagnostic character clines generally form concordant clusters, is also evident in the Tulancingo transect. Chromosome pairs 1, 2, and 6 show strikingly similar patterns in both the shape and position (between localities 4 and 6) of their transitions (Figs. 3–4). The same patterns are also evident in both nondiagnostic chromosomal markers (pairs 3 and 4), although pair 4 segregates fission and pericentric inversion polymorphisms in races FM2 and F5, respectively, and pair 3 is difficult to use as a diagnostic marker because of its similarity in size and morphology to pair 4. Consequently, the curves for these markers plotted in Figure 3 must be interpreted with caution.

The third generality described above concerns the frequently observed discordance between patterns of mtDNA variation and those of nuclear markers collected from the same hybrid zones. Several early studies revealed patterns of extreme discordance between nuclear and cytoplasmic markers (briefly summarized by Harrison et al., 1987; see also Marchant, 1988; Dowling and Hoeh, 1991), and later studies have demonstrated cases in which discordance on a very large scale (several hundred km) likely reflects an apparent "track" of selectively neutral mtDNA markers that trace historical shifts in positions of some zones (e.g., Marchant et al., 1988; Shaw et al., 1990; and Dowling and Hoeh, 1991). Other recent studies, however, have revealed a number of examples of strong concordance between mtDNA and nuclear markers (Szymura et al., 1985; Nelson et al., 1987; Baker et al., 1989). Figure 7 shows a relatively sharp transition from the F5 to FM2 mitochondrial types between localities 4 and 6, but in contrast to the diagnostic chromosomal markers, the mtDNA curve is shifted toward the FM2 race, suggesting introgression of F5 mtDNA into the FM2 race. Inadequate sample sizes make this suggestion simply a hypothesis rather than a firm conclusion, but inspection of the genotypes of animals from locality 5 (Table 3) shows that all animals with hybrid index scores of 3, including the two F_1 hybrids, carry F5 mtDNA, as does the single animal with a score of 1. In contrast, only a single animal with a high score (i.e., an F5-like karyotype) carries FM2 mtDNA (CAP 2028, locality 5, Table 3). If additional sampling supports this pattern, then slight asymmetry in the direction of F5 mtDNA introgression would be confirmed. Takahata and Slatkin (1984) have shown mathematically that neutral mtDNA markers can easily flow across species boundaries, but mtDNA must coevolve in a nuclear background because it does not encode all necessary enzymes for replication, transcription, and translation and it encodes only some protein subunits. Consequently, its function may be strongly dependent upon its nuclear background (reviewed by Moritz et al., 1987). The asymmetry in the direction of F5 mtDNA introgression in the Tulancingo transect, therefore, may be due to either asymmetries of mate choice in which F5 females mated preferentially with FM2 males or asymmetrical selection re-

sulting from incompatibility between the FM2 mitochondrial genome on an F5 nuclear background (theoretical considerations presented in Asmussen et al., 1987). We cannot, at present, choose between these alternatives, but allozyme-based genetic distances and rDNA restriction site variation between the F5 and FM2 cytotypes suggest low levels of nuclear divergence and similar genetic backgrounds between the two. However, asymmetrical mtDNA introgression due to preferential mate choice is well documented in other vertebrates (e.g., Lamb and Avise, 1986) and seems potentially likely between at least some combinations of chromosome races in the *S. grammicus* complex (see Discussion in Arévalo et al., 1993). Regardless, the overall shift depicted in Figure 7 suggests virtual absence of introgression in either direction beyond the transitional areas.

Patterns of ribosomal-DNA variation in the Tulancingo zone.—Figure 7 reveals substantially more introgression of rDNA markers across the transect, relative to other diagnostic markers (chromosomes and mtDNA), but it is largely asymmetrical. The rDNA type-B repeat, diagnostic of the FM2 cytotype, is present at all other localities east of site 6; it is the dominant type at sites 5, 4, and 2,3 but is present only as a single A + B heterozygote in Tulancingo (Table 3). The distribution of all three classes of repeats at localities 2,3 and 4 suggests that it is behaving as an independent locus. For example, individuals may be pure F5 as designated by their karyotypes (i.e., they have hybrid index scores of 6), yet carry the FM2 type-B rDNA repeat (CAP 2041 at site 4, Table 3) or be heterozygous for both types (A + B, as in CAP 2004 at locality 5, Table 3). Further, the two F_1 hybrids (2005 and 2012 at locality 5) are not heterozygous for the rDNA repeat (both carry type-B in this case). The ribosomal-DNA repeats are mid- to highly repetitive sequences arranged in tandem arrays in the nucleolar organizer regions (NORs) of vertebrate chromosomes and, consequently, may be located on one or more chromosome pairs. In all races of *S. grammicus* that have been examined, the NORs are located at the telomeric ends of the long arms of chromosome pair 2 (reviewed in Sites et al., 1992). Because they are confined to a single pair, they should be inherited as a single-copy nuclear marker unless crossing over occurs within the repeats. The distribution of rDNA repeats across the Tulancingo transect shows that they behave as markers unlinked to pair 2 and introgress into F5 populations adjacent to the hybrid zone. This marker provides independent support for the chromosomal markers that reveal backcrossing at locality 5, because repeated backcrossing is needed for substantial introgression. Sample sizes are inadequate at present to determine whether the apparent absence of introgression of F5 rDNA into the FM2 cytotype is real. Results from the companion study by Arévalo et al. (1993) suggest that there is no a priori reason to expect one way introgression at this marker. Well-documented cases of extensive rDNA introgression are known in plants (Arnold et al., 1990), and ongoing population genetic studies of the *S. grammicus* complex will provide a more extensive characterization of rDNA genotype diversity within both the F5 and FM2 cytotypes.

Genetic structure of the Tulancingo hybrid zone.—There are two general patterns for the classes of markers examined across the Tulancingo transect. The most conspicuous is the previously discussed limited introgression/concordance of clinal shape for some characters (the diagnostic chromosome pairs, FM2 mtDNA types, rDNA, and DS and SDL meristic traits). The second is the significant level of geographically structured variation for other characters in which patterns of frequency shifts are independent of the character clines defining the zone. Included in this category are most of the allozyme loci, some F5 mtDNA haplotypes, and several meristic traits. For example, the allozyme frequency shifts plotted in Figure 5 show both heterogeneity in individual markers across the transect, as well as between-locus heterogeneity in patterns. Patterns are similar for Iddh-A(b) and αMan-A(b), but these two differ from the other three loci, and the latter differ among themselves. Because none of these markers is diagnostic for either of these cytotypes (E. Arévalo and JWS, unpubl. data), we cannot unequivocally interpret any of these patterns as evidence of reduced gene flow. Our estimates of population genetic structure across all samples and loci indicate strong subdivision, which would restrict the potential for rapid gene flow unless episodes of extinction and recolonization were frequent. The distributions of mtDNA haplotype frequencies reveal similar patterns (Tables 5, 7), and are significantly different from expectations of panmixia in the Monte Carlo simulations ($\chi^2 = 90.7$, $P < 0.001$ in 1000 simulations). The fact that the shapes of the allozyme and mtDNA clines are neither concordant with each other nor with the diagnostic markers implies that the patterns result from either historical influences (inheritance of ancestral polymorphisms, zonal shifts in response to climatic

changes, etc.) and/or current population parameters.

For example, if allozyme markers are selectively neutral and not tightly linked to any markers for which selection strongly influences clinal shape, they would be expected to pass freely through the zone in either direction (Barton and Hewitt, 1989). However, the rate at which neutral alleles move across such a zone depends upon the age of the contact and the structure of the populations. Until ongoing population genetic and phylogenetic studies are complete, we cannot address the issues of historical influences, but the earlier population cytogenetic studies of this complex by Arevalo et al. (1991) provided evidence for the overriding influence of drift on the local frequencies of neutral chromosomal rearrangements. Population structures will be influenced by migration patterns and rates from different source populations, and local differences in neighborhood sizes (Sokal and Wartenberg, 1981). We have no information on these parameters for any populations of *S. grammicus*, but in the course of our field work, we have frequently noticed differences in habitat quality and densities of lizards. These observations suggest that at least some of the above parameters vary over time and space and, thus, likely influence population structure. All of these factors are likely compounded by anthropogenic activity in the Tulancingo Valley, as *Agave* and building materials are frequently moved from place to place in the course of agricultural activities. This may facilitate gene flow, but it would also likely alter densities (temporary destruction of local habitats, by cutting *Agave*, for example) and colonization from source populations that would not serve as sources for long-distance movement without human intervention. We conclude that the allozyme and F5 mtDNA haplotype frequency data reflect a neutral diffusion/isolation-by-distance process (Wright, 1943) in which sampling error strongly influences short-term gene flow patterns.

One unexpected finding was the presence of deep mtDNA divergence within the F5 cytotype. The haplotypes identified as F5-I and F5*-VII differ by at least one restriction site in 10 of the 11 enzymes that revealed variation across the Tulancingo transect (Table 4). There were a total of seven different F5 haplotypes (F5-I–VI, and F5*-VII, Table 5), with d_r values ranging up to 0.106 (between F5-V and F5*-VII, Table 5). In contrast, only two haplotypes were identified in FM2 (types VIII and IX, Table 5, although these were determined from a survey of only seven animals), and these differed by a d_r of 0.003 (Table 6). Nevertheless, the high haplotype diversity (seven of nine total haplotypes) and deep division (F5 vs F5*) within the F5 cytotype was unexpected. This suggests that this race may be either introgressed with mitochondrial genomes from other, as yet unidentified, donor populations or that it has originated only very recently from a highly polymorphic ancestral population and has not yet completed the stochastic lineage sorting necessary for coalescence of mitochondrial and nuclear gene genealogies (Neigel and Avise, 1986; Pamilo and Nei, 1988; Avise, 1989). With respect to the possibility of introgression, we emphasize the point that all F5 animals collected at locality 1 were on or immediately adjacent to humanmade structures (rock walls, houses, etc.) in the city of Tulancingo. This may be significant because the LS (=low standard) cytotype is known from such habitats in other localities, although we have not collected this race in Tulancingo. If present, however, this race could provide a source of mtDNA introgression into the F5 race, and we note that the very distinct F5*-VII haplotype is restricted to this single locality (Table 5).

A third possibility is that the F5 cytotype is extremely old relative to FM2 and that the haplotype diversity within it simply reflects splitting of daughter lineages over time within what is otherwise a monophyletic unit as defined by nuclear markers. However, previous studies of other cytotypes within the *S. grammicus* complex have shown that races hypothesized to be relatively basal to the entire radiation can usually be distinguished from more derived races (i.e., FM2) by several fixed differences at allozyme, mtDNA, or rDNA markers (Sites and Davis, 1989). Thus, given the levels of mtDNA diversity uncovered in this survey, we would also expect to find higher levels of divergence in both classes of nuclear markers. The data presented in this paper do not permit us to choose among these possibilities, but widespread geographic screening efforts are now in progress that will clarify these issues.

Morphological variation across the Tulancingo hybrid zone.—Similar to the allozyme markers, the meristic traits do not generally show sharp breaks between localities 4 and 6. There are clear clinal shifts in numbers of dorsal spots and subdigital lamellae (Table 8), but other characters either show zigzag shifts or, in the case of infralabials (INL), an increase in mean numbers in the center of the transect, trailing off toward either end. This may be due to the influence of hybridization itself, because natural

hybridization is known to touch off bursts of mutational activity in single-copy nuclear markers (Shaw et al., 1983; Woodruff, 1989), but the expected outcome of hybridization on individual morphological traits is not clear.

In an experimental study of morphological consequences of hybridization between four combinations of different genera of cyprinid fishes (*Semotilus* × *Campostoma*, *Semotilus* × *Clinostomus*, *Semotilus* × *Nocomis*, and *Semotilus* × *Rhinichthys*), Ross and Cavender (1981) showed that F_1 individuals were generally intermediate between parental taxa with respect to their hybrid index scores, but the majority of individual characters were not intermediate. These results were obtained for the majority of characters in three of the four crosses, and Ross and Cavender suggested that such patterns were likely due to the fact that most morphological traits used in such studies were probably random samples of two classes of traits. First, some morphological characters are probably controlled by one or very few loci in which certain alleles or loci dominate the phenotypic expression of alternate markers in hybrid genomes. In these cases, expression of such traits in F_1 animals is shifted toward the parent having the dominant marker. The second class of traits represents those controlled by complex epistatic, polygenic systems, and, in this case, the influence of regulatory or modifier loci could strongly bias the direction of character expression if the trait has little additive inheritance. Overall levels of morphological variability are also expected to be generally higher in animals of mixed parentage (see Dessauer and Cole, 1991). Thus, results of multivariate analyses of a mixture of these kinds of traits would likely show overall morphological intermediacy if roughly equal numbers of traits showed skewed patterns of expression in opposite directions, and few specific characters would show clear intermediacy.

In this study, the multivariate analyses showed that the two parental cytotypes are distinct, but all classes of hybrid and backcross individuals phenotypically overlap with parentals, especially the pure FM2 (Fig. 8). Morphological data alone, therefore, do not offer the resolution necessary to determine the detailed genetic structure of this contact, although they are useful for assessing general morphological intermediacy of hybrid and backcross classes of animals (Fig. 9). One other possibility that can not be excluded at this point is that the fine-scale microgeographic structure of this zone may be a patchy mosaic, which would not be apparent at the sampling scale of our single linear transect. Rand and Harrison (1989) described a recent example in a hybrid zone between two species of the cricket genus *Gryllus* in which both allozyme and morphological characters displayed an extremely patchy, zigzag distribution that was tightly correlated with soil type. This possibility can only be addressed in *S. grammicus* by intensive, fine-scale sampling efforts.

Origin and maintenance of the Tulancingo hybrid zone.—Determination of hybrid zone origins has proven extremely difficult in most cases because many of the processes involved in the origin of primary divergence versus secondary contact will produce identical patterns. Further, there is usually insufficient information for reconstruction of relevant historical and ecological factors that determine the current distributions of hybridizing populations (Hewitt, 1988; Barton and Hewitt, 1989; Harrison, 1991). Two lines of evidence, however, may permit rejection of some of the possibilities. For example, formation of primary zones of intergradation by definition must occur in the absence of geographic separation and result from strong selection favoring alternative character states at a given locus in two different environments. Thus, two alternative stable equilibria are maintained along an environmental gradient, and the steepness of the clinal transition between them reflects the abruptness of transition from one environment to the other (Barton and Hewitt, 1989). Some have proposed that sufficient selection along such gradients may lead to speciation (the parapatric model of Endler, 1977, and others). Two obvious expectations from this process are that (1) the lineages involved in the contact must be sister species; and (2) there should be tight correlations between one or more characters with one or more environmental variables to which the alternative states represent adaptations. In contrast, secondary contacts may or may not be associated with ecological transitions and can form between sister or nonsister taxa.

With respect to the Tulancingo hybrid zone, there is no obvious single environmental or habitat shift from across the transect. Vegetational differences are found at the extreme eastern and western edges (Table 1); the F5 population sampled in the town of Tulancingo was taken from cactus (*Opuntia*) and rock walls, and the FM2 animals at the other end were all collected from rows of *Agave*. However, in the transitional localities, F5 individuals are taken from all kinds of habitats (walls, stone fences, pine and oak trees, rows of *Agave*, and *Opuntia*), and FM2 individuals are found on all of the same structures. The single exception is that FM2 animals

seem to occur less frequently on trees, but this is a subjective impression that will require larger sample sizes and quantification to verify. The entire valley through which populations were sampled has been intensively modified by small-scale agriculture, and reconstruction of the composition of the original vegetation may be impossible without palynological data. The climate is generally more mesic from Tulancingo eastward and more xeric from Matias Rodriguez westward, but the transition (again as subjectively evaluated) appears to be rather broad (25–35 km). There is no conspicuous habitat or other environmental variable correlated with the dramatic shift from F5 to FM2 karyotypes in the center of the transect.

The absence of an obvious environmental parameter, of course, does not preclude its existence, but we believe that other data strongly support the secondary contact hypothesis for the origin of this zone. The concordant shifts between the three diagnostic chromosomal markers, and between these and the mtDNA haplotypes (with caveats described above), suggest that the F5 and FM2 cytotypes accumulated some divergence in isolation from each other and that the sharp boundary at the point of transition between them defines a secondary contact. Similar findings of concordant shifts in a number of unlinked markers in other organisms (Szymura and Barton, 1991; Good, 1989; Dowling and Hoeh, 1991) have been interpreted to reflect secondary contact, because it is unlikely that selection along environmental gradients would produce identical cline shapes in several independent markers, even allowing for the effects of linkage disequilibrium (Barton and Hewitt, 1989). The phylogenetic position of the F5 cytotype is unknown, but a previous molecular study by Sites and Davis (1989) confirmed the derived status of FM2 relative to six other cytotypes. (F5 was not included in that study because it was at the time extremely poorly documented in central Mexico.) Molecular studies currently in progress will extend the work of Sites and Davis (1989) by including additional mtDNA markers (sequences) and additional populations of all central Mexico chromosome races. Demonstration of a nonsister relationship between the F5 and FM2 cytotypes would confirm the hypothesis of secondary contact for the origin of the Tulancingo transect.

We do not have data bearing directly on the question of maintenance of this hybrid zone, other than the aforementioned absence of a visible environmental gradient. In addition to maintenance due to selection favoring direct response of genotypes to environmental variation, hybrid zones are also maintained by a balance between dispersal and intrinsic inferiority of the hybrids themselves. Inferiority of the hybrids may in turn result from direct selection against particular hybrid 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 (Harrison, 1991). We cannot presently evaluate these alternatives and here make only a few comments.

First, all three of these possibilities are based on hybrid unfitness as an intrinsic property of the hybrid genotypes themselves, so that fitness values themselves should be independent of the environment. In these cases, hybrid zones may form and stabilize in regions of environmental homogeneity (they appear to settle in "troughs" of low density, Barton and Hewitt, 1989), which is consistent with our subjective impression of both the Ajusco and Tulancingo transects. Second, of the possible causes of selection against the hybrids, we suspect that the chromosomal differences between the F5 and FM2 races are likely contributors in the Tulancingo transect (see reviews of mechanisms in Sites and Moritz, 1987; Searle, 1988), whereas epistatic interactions and/or frequency-dependent selection against rare alleles may predominate in the Ajusco transect. The extent of chromosomal polytypy in the *S. grammicus* complex, and the degree of subdivision within it by hybrid zones of different degrees of chromosomal complexity, offer unique opportunities for studies designed to separate these components of hybrid unfitness. Microgeographic sampling and cytogenetic studies currently in progress will test the assumption of negative heterosis for chromosomal rearrangements in the Tulancingo transect.

Acknowledgments

We thank personnel of the Direccion General de la Flora y Fauna Silvestres for scientific collecting permits issued for these studies and G. Casas-A. and F. Rivera for logistical support in Mexico. Field and laboratory assistance was provided by A. Aguilar, J. Camarillo, J. Contreras, H. Gadsden, A. Gonzalez, C. Harrison, A. Jeffery, L. Javier, E. Jimenez, J. Juarez, C. Lopez, C. Lorenzo, M. Mancilla, F. Mendez, F. Mendoza, A. Nieto, C. Porter, A. Rimirez, J. Sites, and H. Sites. Financial support was provided by research grants from the M. L. Bean Life Science Museum and the Professional Development Committee, both of Brigham Young Uni-

versity, the National Geographic Society (grant no. 3088–85), and the National Science Foundation (grants BSR 85–09092 and 88–45298). We thank T. Dowling for the UPGMA algorithm and T. Dowling and K. Reed for reading earlier drafts of this manuscript, L. Richardson and J. R. Gold for assistance with the REAP software, and E. Arevalo for preparing the Resumen.

Literature Cited

Arévalo, E., G. Casas, S. K. Davis, G. Lara, and J. W. Sites, Jr. 1993. Parapatric hybridization between chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae). I. Structure of the Ajusco transect. Copeia 1993:320–390.

———, C. A. Porter, A. Gonzalez, F. Mendoza, J. L. Camarillo, and J. W. Sites, Jr. 1991. Population cytogenetics and evolution of the *Sceloporus grammicus* complex (Iguanidae) in central Mexico. Herp. Monogr. 5:79–115.

Arnold, M. L., B. D. Bennett, and E. A. Zimmer. 1990. Natural hybridization between *Iris fulva* and *Iris hexagona*: pattern of ribosomal DNA variation. Evolution 44:1512–1521.

Asmussen, M. A., J. Arnold, and J. C. Avise. 1987. Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes. Genetics 115:755–768.

Avise, J. C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution 43:1192–1208.

Baker, R. J., S. K. Davis, R. D. Bradley, M. J. Hamilton, and R. A. Van Den Bussche. 1989. Ribosomal DNA, mitochondrial DNA, chromosomal and allozymic studies of a contact zone in the pocket gopher, *Geomys*. *Ibid.* 43:63–75.

Barton, N. H. 1983. Multilocus clines. *Ibid.* 37:454–471.

———, and G. M. Hewitt. 1985. Analysis of hybrid zones. Ann. Rev. Ecol. Syst. 16:113–148.

———, and ———. 1989. Adaptation, speciation and hybrid zones. Nature 341:497–503.

Buth, D. G., and R. W. Murphy. 1990. Appendix 1: Enzyme staining formulas, and Appendix 2: Buffers and tracking dye for isozyme electrophoresis, p. 99–126. *In:* Molecular systematics. D. M. Hillis and C. Moritz (eds.). Sinauer Assoc., Inc., Sunderland, Massachusetts.

Dessauer, H. C., and C. J. Cole. 1991. Genetics of whiptail lizards (Reptilia: Teiidae: *Cnemidophorus*) in a hybrid zone in southwestern New Mexico. Copeia 1991:622–637.

Dowling, T. E., and W. R. Hoeh. 1991. The extent of introgression outside the contact zone between *Notropis cornutus* and *Notropis chrysocephalus* (Teleostei: Cyprinidae). Evolution 45:944–956.

———, G. R. Smith, and W. M. Brown. 1989. Reproductive isolation and introgression between *Notropis cornutus* and *Notropis chrysocephalus* (Family Cyprinidae): comparison of morphology, allozymes, and mitochondrial DNA. *Ibid.* 43:620–634.

Endler, J. A. 1977. Geographic variation, clines, and speciation. Princeton Univ. Press, Princeton, New Jersey.

Good, D. A. 1989. Hybridization and cryptic species in *Dicamptodon* (Caudata: Dicamptodontidae). Evolution 43:728–744.

Hall, W. P., and R. K. Selander. 1973. Hybridization of karyotypically differentiated populations in the *Sceloporus grammicus* complex (Iguanidae). *Ibid.* 27:226–242.

Harrison, R. G. 1991. Hybrid zones: windows on evolutionary process, p. 69–128. *In:* Oxford surveys in evolutionary biology. Vol. 7. D. Futuyma and J. Antonovics (eds.). Oxford Univ. Press, London, England.

———, D. M. Rand, and W. C. Wheeler. 1987. Mitochondrial DNA variation in field crickets across a narrow hybrid zone. Mol. Biol. Evol. 4:144–158.

Hewitt, G. M. 1988. Hybrid zones—natural laboratories for evolutionary studies. Trends Ecol. Evol. 3:158–167.

International Union of Biochemistry. Nomenclature Committee. 1984. Enzyme nomenclature 1984. Academic Press, Orlando, Florida.

Kerster, W. 1964. Neighborhood size in the rusty lizard, *Sceloporus olivaceus*. Evolution 18:444–457.

Lamb, T., and J. C. Avise. 1986. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. Proc. Nat. Acad. Sci. U.S. 83:2526–2530.

Levene, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20:91–94.

Marchant, A. D. 1988. Apparent introgression of mitochondrial DNA across a narrow hybrid zone in the *Caledia captiva* species-complex. Heredity 60: 39–46.

———, M. L. Arnold, and P. Wilkinson. 1988. Gene flow across a chromosomal tension zone. I. Relicts of ancient hybridization. *Ibid.* 61:321–328.

McElroy, D., P. Moran, E. Bermingham, and I. Kornfield. 1991. REAP. The restriction enzyme analysis package. Version 4.0. Center for Marine Studies, Univ. of Maine, Orono.

Moritz, C., T. E. Dowling, and W. M. Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Ann. Rev. Ecol. Syst. 18:269–292.

Neff, N. A., and G. R. Smith. 1979. Multivariate analysis of hybrid fishes. Syst. Zool. 28:176–196.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

———. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York, New York.

———, and J. C. Miller. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. Genetics 125:873–879.

———, and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. *Ibid.* 97: 145–163.

———, and ———. 1983. Maximum likelihood es-

timation of the number of nucleotide substitutions from restriction site data. *Ibid.* 105(1):207–217.

NEIGEL, J. E., AND J. C. AVISE. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation, p. 515–534. *In:* Evolutionary processes and theory. E. Nevo and S. Karlin (eds.). Academic Press, New York, New York.

NELSON, K., R. J. BAKER, AND R. L. HONEYCUTT. 1987. Mitochondrial DNA and protein differentiation between hybridizing cytotypes of the white-footed mouse, *Peromyscus leucopus.* Evolution 41: 864–872.

PAMILO, P., AND M. NEI. 1988. Relationships between gene trees and species trees. Mol. Biol. Evol. 5:568–583.

PORTER, C. A., AND J. W. SITES, JR. 1986. Evolution of the *Sceloporus grammicus* complex (Sauria: Iguanidae) in central Mexico. Population cytogenetics. Syst. Zool. 35:334–358.

RAND, D. M., AND R. G. HARRISON. 1989. Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. Evolution 43:432–449.

ROFF, D. A., AND P. BENTZEN. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. Mol. Biol. Evol. 6:539–545.

ROSS, M. R., AND T. M. CAVENDER. 1981. Morphological analyses of four experimental intergeneric cyprinid hybrid crosses. Copeia 1981:377–387.

SEARLE, J. B. 1988. Selection and Robertsonian variation in nature: the case of the common shrew, p. 507–531. *In:* The cytogenetics of mammalian autosomal rearrangements. A. Daniel (ed.). A. R. Liss, New York, New York.

SHAW, D. D. 1981. Chromosomal hybrid zones in orthopteroid insects, p. 146–170. *In:* Evolution and speciation. W. R. Atchley and D. Woodruff (eds.). Cambridge Univ. Press, London, England.

———, A. D. MARCHANT, M. L. ARNOLD, N. CONTRERAS, AND B. KOHLMANN. 1990. The control of gene flow across a hybrid zone: a selective role for chromosomal rearrangement? Can. J. Zool. 68:1761–1769.

———, P. WILKINSON, AND D. J. COATES. 1983. Increased chromosomal mutation rate after hybridization between two subspecies of grasshoppers. Science 220:1165–1167.

SITES, J. W., JR., AND S. K. DAVIS. 1989. Phylogenetic relationships and molecular variability within and among six chromosome races of the *Sceloporus grammicus* complex (Sauria, Iguanidae), based on nuclear and mitochondrial markers. Evolution 43:296–317.

———, AND I. F. GREENBAUM. 1983. Chromosome evolution in the iguanid lizard *Sceloporus grammicus.* II. Allozyme variation. *Ibid.* 37:54–65.

———, AND C. MORITZ. 1987. Chromosomal evolution and speciation revisited. Syst. Zool. 36:153–174.

———, AND R. W. MURPHY. 1991. Isozyme evidence for independently derived, duplicate G3PDH loci in squamate reptiles. Can. J. Zool. 69:1281–1296.

———, J. W. ARCHIE, C. J. COLE, AND O. FLORES-VILLELA. 1992. A review of phylogenetic hypotheses for the lizard genus *Sceloporus* (Phrynosomatidae): implications for ecological and evolutionary studies. Bull. Amer. Mus. Nat. Hist. 213: 1–110.

———, J. L. CAMARILLO, A. GONZALES, F. MENDOZA, L. JAVIER, M. MANCILLO, AND G. LARA-GONGORA. 1988. Allozyme variation and genetic divergence within and between three cytotypes of the *Sceloporus grammicus* complex (Sauria, Iguanidae) in central Mexico. Herpetologica 44:297–307.

SOKAL, R. R., AND D. W. WARTENBERG. 1981. Space and population structure, p. 186–213. *In:* Dynamic spatial models. D. Griffith and R. McKinnon (eds.). Sijthoff and Noordhuff, Amsterdam, The Netherlands.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281–283.

SZYMURA, J. M., AND N. H. BARTON. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*: comparisons between transects and between loci. Evolution 45:237–261.

———, C. SPOLSKY, AND T. UZZELL. 1985. Concordant changes in mitochondrial and nuclear genes in a hybrid zone between two frog species (genus *Bombina*). Experientia 41:1469–1470.

TAKAHATA, N., AND M. SLATKIN. 1984. Mitochondrial gene flow. Proc. Nat. Acad. Sci. U.S. 81:1764–1767.

TINKLE, D. W. 1965. Population structure and effective size of a lizard population. Evolution 19: 569–573.

WALSH, J. B. 1982. Rate of accumulation of reproductive isolation by chromosome rearrangements. Am. Nat. 120:510–532.

WEIR, B. S. 1990. Genetic data analysis. Sinauer Assoc., Inc., Sunderland, Massachusetts.

———, AND C. C. COCKERHAM. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

WOODRUFF, D. S. 1989. Genetic anomalies associated with *Cerion* hybrid zones: the origin and maintenance of new electromorphic variants called hybrizymes. Biol. J. Linn. Soc. 36:281–294.

WRIGHT, S. 1943. Isolation by distance. Genetics 28: 114–138.

(JWS, DWH, AND BAM) DEPARTMENT OF ZOOLOGY, BRIGHAM YOUNG UNIVERSITY, PROVO, UTAH 84602; (SKD) DEPARTMENT OF ANIMAL SCIENCE, TEXAS A&M UNIVERSITY, COLLEGE STATION, TEXAS 77843; PRESENT ADDRESSES: (DWH) DEPARTMENT OF BIOLOGY, WASHINGTON UNIVERSITY, ST. LOUIS, MISSOURI 63130; AND (GL) CENTRO ECOLOGICO DE SONORA, A.P. 1497, HERMOSILLO, SONORA, MEXICO. Submitted 8 Nov. 1991. Accepted 15 March 1992. Section editor: D. G. Buth.