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MITOCHONDRIAL DNA SEQUENCE DIVERGENCE AND PHYLOGENETIC RELATIONSHIPS AMONG EIGHT CHROMOSOME RACES OF THE *SCELOPORUS GRAMMICUS* COMPLEX (PHRYNOSOMATIDAE) IN CENTRAL MEXICO

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Abstract.—A 2,479-base pair mitochondrial DNA fragment was sequenced for eight chromosome races of *Sceloporus grammicus* from central Mexico to estimate their phylogenetic relationships. The species *S. poinsetti* and *S. olivaceus* were used separately as alternative outgroups. A total of 795 positions varied in three complete protein-coding genes examined (ND3, ND4L, ND4), and 52 of 292 positions varied across five transfer RNAs examined (glycine, argenine, histidine, serine, leucine). Sequence divergence values ranged from 0.0 to 0.23 among the ingroup taxa, and a maximum of 0.26 was observed between ingroup and outgroup taxa. Alternative analyses based upon equally weighted characters and several alternative character-weighting options were used to obtain phylogenetic hypotheses for the complex, and a single most-parsimonious tree was selected from among these on the basis of a new character-weighting method that takes into account the observed frequencies of all 12 possible substitutions for protein genes. The most-parsimonious cladogram showed that chromosomal evolution in this complex has been more complicated than previously hypothesized. Several rearrangements (Robertsonian fissions) have evolved independently on two or more occasions, which corroborates evidence from other studies showing that single rearrangements are not underdominant upon their origin, and their fixation probabilities are enhanced by repeated origins. These observations refute expectations of some general models of chromosome evolution. The same phylogenetic hypothesis was used to test the minimum-interaction model of chromosome evolution and a specific model for the evolution of macrochromosome 2. A clear distinction was also possible among alternative hypotheses of relationship for three chromosome races involved in hybridization, and the consequences for the role of chromosomal rearrangements in reducing gene flow are discussed in this context. [Mitochondrial DNA; *Sceloporus grammicus*; molecular phylogeny; chromosome evolution; hybrid zones.]

Resumen.—Un fragmento de 2,479 pares de bases del ADN mitocondrial en ocho razas cromosómicas de *Sceloporus grammicus* de la porción central de México fue secuenciado con el fin de estimar sus relaciones filogenéticas. Las especies *S. poinsetti* y *S. olivaceus* fueron usadas como grupos externos alternativos. Un total de 795 posiciones variaron en tres genes completos que codifican para proteinas (ND3, ND4L, y ND4) y 52 de las 292 posiciones variaron a través de los cinco tARNs examinados (glicina, argenina, histidina, serina, y leucina). El rango de valores de divergencia en la secuencia fue de 0.0 a 0.23 entre los taxa del complejo y un máximo de 0.26 se observó entre los miembros del complejo y los taxa externos. Análisis alternativos basados en caracteres con pesos equivalentes y con diferentes opciones de pesos en los caracteres fueron usados para obtener hipótesis filogenéticas del complejo, y un único árbol con la mayor parsimonia fue seleccionado de entre estos árboles con base en un nuevo método de pesos de los caracteres, que toma en consideración las frecuencias observadas de los 12 posibles sustituciones para los genes de proteinas. El cladograma con mayor parsimonia mostró que la evolución cromosómica en este complejo ha sido mas complicada que como ha sido explicada con anterioridad. Varios rearreglos (fisiones Robertsonianas) han evolucionado independientemente en dos o más ocasiones, lo cual corrobora la evidencia de otros estudios que muestran que rearreglos únicos no son menos dominantes desde su origen y que sus probabilidades de que sean fijados son incrementadas por los orígenes repetidos. Estas observaciones refuten lo esperado en algunos modelos generales de la evolución cromosómica. La misma hipótesis filogenética fue usada para probar el modela de la interacción mínima de evolución cromosómica y un modelo específico

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para la evolución del cromosoma 2. Fue posible obtener una distinción clara entre posibles relaciones alternas para las tres razas cromosómicas involucradas en la hibridización y en este contexto, las consecuencias que el papel de los rearreglos cromosómicos tienen en reducir el flujo génico fueron discutidos.

The *Sceloporus grammicus* complex (Phrynosomatidae; Frost and Etheridge, 1989) has been studied extensively for the last two decades because of its extreme chromosomal polytypy and the possible association between chromosomal divergence and speciation potential (White, 1978; King, 1993). Distributional studies have shown the complex to consist of multiple chromosomal races, or cytotypes, with diploid ($2n$) numbers ranging from 32 up to 46 and a geographic distribution extending from extreme southern Texas through most of mainland Mexico (Hall, 1973; Porter and Sites, 1986; Sites et al., 1987; Arévalo et al., 1991). At least seven hybrid zones have been identified in central Mexico, and these involve contacts between six different combinations of chromosome races (Arévalo et al., 1993).

Chromosomal evolution in this group was hypothesized to have occurred via the successive fixation of chromosomal fissions to produce a linear series of cytotypes, from the ancestral $2n = 32$ (with six large metacentric chromosomes) through all intermediate diploid numbers to the nearly all acrocentric $2n = 46$ race (Hall, 1980, 1983). This hypothesis yields two equally parsimonious cladograms for relationships among these races that differ from each other only with respect to the order of fixation of macrochromosomes 5 and 6 (Fig. 1). If chromosome 5 fissioned first, the following order of fissions would have had to occur to derive a linear series of higher diploid numbers: F5 → F6 → F2 (first rearrangement) → F3 (and establishment of F1 and F4 polymorphisms) → F1 (fixation) and F2 (second mutation, with F4 retained as a polymorphism in the FM2 race). This hypothesis (Fig. 1a) would require either an independent derivation of fission 6 to establish the F6 race near the base of the radiation (as shown) or a refusion of one or more of the other autosomes, which could derive the F6 race at any point in the genealogy after the chromosome 6 fission was first established. The arrangement of the F6 race as depicted in Figure 1a allows for the possibility that some of the known hybrid zones might represent contacts between sister taxa (LS × F6 or HS × F6; see Arévalo et al. [1993] and Hall and Selander [1973] for details). The alternative arrangement (Fig. 1b) simply reverses the order of fissioning chromosomes 5 and 6 at the base of the radiation (and provides several alternatives for the origin of the F5 race) and would indicate that all known hybrid zones are between nonsister taxa.

Various assumptions and corollaries of several classes of chromosomal speciation hypotheses have been previously addressed in the *S. grammicus* complex, including population cytogenetics and enumeration of within-race Robertsonian and inversion autosomal heteromorphisms (Sites, 1983; Porter and Sites, 1986; Arévalo et al., 1991), the meiotic consequences of chromosomal heterozygosity in nonhybrid zone contexts (Porter and Sites, 1985, 1987), the application of microspreading/electron micrographic techniques to evaluate the earliest meiotic stages of these same processes (Reed et al., 1992a, 1992b, 1992c), independent estimates of population structure from single-copy nuclear markers (isozymes) in both comparative (Thompson and Sites, 1986) and computer simulation (Sites et al., 1988b) contexts, morphological and isozyme-based studies of patterns of divergence and their geographic concordance with chromosome markers (Sites, 1982; Lara-Góngora, 1983; Sites and Greenbaum, 1983; Sites et al., 1988a), and several studies characterizing the general structure of some zones of parapatric hybridization (Hall and Selander, 1973; Arévalo et al., 1993; Sites et al., 1993). The chromosomal phylogeny was first independently tested by an isozyme and restriction-site mapping study based on nuclear ribosomal DNA (rDNA) and

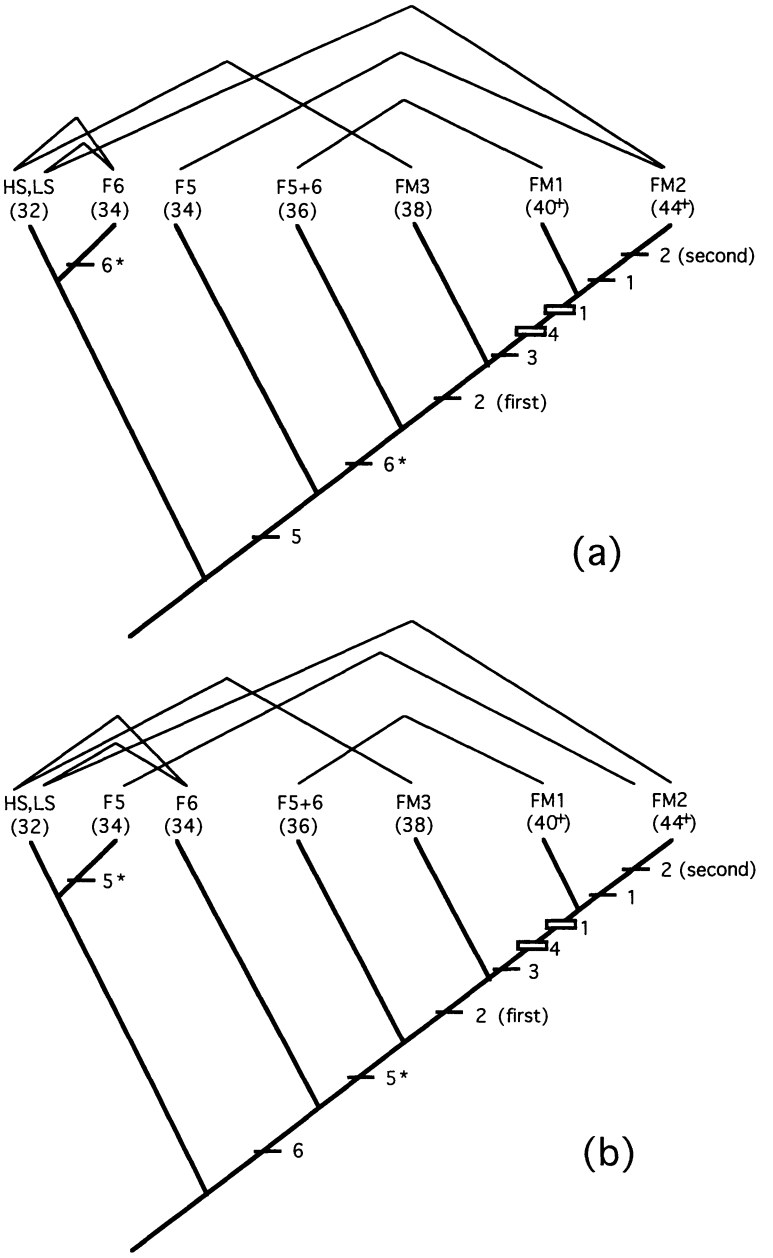

FIGURE 1. Alternative hypotheses for derivation of linear "cascade" of *Sceloporus grammicus* chromosome races (Hall, 1980, 1983). Two of these cytotypes have a $2n = 32$ karyotype (LS and HS), two have $2n = 34$ (F6 and F5), and one each have $2n = 36$ (F5+6), $2n = 38$ (FM3), $2n = 40$–44 (FM1), and $2n = 44$–46 (FM2) cytotypes (Reed et al., 1992c). The solid and open rectangles represent fixed and polymorphic fission rearrangements, respectively, of macrochromosome pairs 1–6. The asterisk indicates independent fixations of the same chromosome required in either hypothesis, i.e., chromosome 6 in (a) and chromosome 5 in (b), and lines connect pairs of races known to form parapatric hybrid zones on the basis of diagnostic chromosome markers (see Arévalo et al., 1991, 1993, for details).

mitochondrial markers (Sites and Davis, 1989), but this study was completed prior to discovery of the F5 race in central Mexico. One purpose of the present study was to extend the earlier work of Sites and Davis (1989) on the basis of mitochondrial DNA (mtDNA) sequences presented here for all cytotypes. These data provide the necessary comparative framework (Brooks and McLennan, 1991; Harvey and Pagel, 1991) for testing several hypotheses of chromosomal evolution.

We had two objectives in this study. First, we evaluated a new character-weighting method for a large amount of mtDNA protein-coding gene sequence relative to several other commonly used weighting schemes. Second, we selected a single best-supported phylogenetic hypothesis to test the alternative patterns of chromosomal evolution depicted in Figure 1. A well-corroborated cladistic hypothesis for the *S. grammicus* complex permits testing of several general aspects of chromosome evolution (i.e., the minimum-interaction hypothesis of Imai et al. [1986]).

We then tested the sequence of chromosomal mutation events proposed by Reed et al. (1992c) for the derivation of the unique morphology of chromosome 2 in the FM2 cytotype. Strong support for almost any alternative to the Reed et al. proposal would have important implications for the molecular structure of eukaryote chromosomes. The alternative hypotheses for relationships among the HS, LS, and F6 races (Fig. 1a vs. Fig. 1b) could also be rigorously evaluated. These three races replace each other vertically along elevational gradients on mountain ranges surrounding Mexico City in the order LS → F6 → HS (lowest to highest elevations). Hybridization occurs at both the LS × F6 and F6 × HS contacts (Hall, 1973; Arévalo et al., 1993), and knowledge of phylogenetic relationships of the populations involved in these (and other) hybrid zones will inform interpretations of the role chromosomal rearrangements play as possible postmating isolating mechanisms. As emphasized by Sites and Moritz (1987), the strongest case for a major contribution by a chromosomal rearrangement to selection against hybrids can be made for a hybrid zone between sister taxa, because complicating factors due to overall genic divergence should be minimal. If genetic divergence is the most important influence on hybrid fitness, its influence should be manifested in comparisons of hybrid zones between distantly related chromosome races, relative to interactions between sister races differing by the same rearrangement. The LS × F6 and F6 × HS contacts provide this kind of comparison.

Materials and Methods

Sampling

Lizards representing all eight cytotypes of the *Sceloporus grammicus* complex (LS, HS, F6, F5, F5+6, FM3, FM1, and FM2) were collected during the summers of 1985, 1986, 1989, and 1991 from different localities on the Mexican Plateau in central Mexico. Representatives from another member of the *S. grammicus* complex, *S. grammicus grammicus* (Smith, 1939) from the Sierra de Igualatlaco of southwestern Mexico (state of Guerrero), an area peripherally isolated from all *S. grammicus* cytotypes on the Mexican Plateau, were also evaluated. Karyotypes were determined for all animals from preparations made from marrow of long bones (Porter and Sites, 1985), and tissues were removed and stored in liquid nitrogen for future use in molecular studies. All individuals were prepared as voucher specimens and deposited in one of four museum collections (Table 1). The lizards used for this study constitute a subsample of the collections mentioned above, with one specimen representing each chromosomal race.

In addition to the eight cytotypes, two other *Sceloporus* species were collected for use as successively more distant outgroups of the *S. grammicus* complex: *S. poinsetti* as the first outgroup ($2n = 32$, identical to that for the HS and LS races of *S. grammicus*), and *S. olivaceus* as the second outgroup ($2n = 22$; phylogenetic hypotheses for the genus were reviewed by Sites et al. [1992]).

TABLE 1. List of cytotypes, diploid numbers, localities, and voucher numbers for the eight central Mexico cytotypes of the *Sceloporus grammicus* complex, *S. g. grammicus* (SGG), and the two outgroups used, *S. poinsetti* (SP) and *S. olivaceus* (SO).

Race	2*n*	Locality[a]	Voucher number[b]
LS	32	San Miguel Ajusco, D.F., Mexico	BYU-38487
HS	32	Presa Iturbide, Mexico, Mexico	EDHEM-0653
F6	34	El Capulín, Mexico, Mexico	BYU-38494
F5	34	Apulco, Hidalgo, Mexico	MZFC-4849
F5+6	36	Vizarrón, Queretaro, Mexico	MZFC-938
FM3	38	Mineral el Chico, Hidalgo, Mexico	MZFC-947
FM1	40	Huichapan, Hidalgo, Mexico	MZFC-940
FM2	46	Ajacuba, Hidalgo, Mexico	BYU-38691
SGG	32	Igualatlaco, Guerrero, Mexico	IBHED-07177
SP	32	Catron Co., NM, USA	BYU-42534
SO	22	Concho Co., TX, USA	BYU-42888

[a] Specific localities were reported by Arévalo et al. (1991).

[b] BYU = M. L. Bean Life Science Museum, Brigham Young University; EDHEM = Ecología de la Herpetofauna del Estado de México, Escuela Nacional de Estudios Profesionales-Iztacala; MZFC = Colección Herpetológica, Museo de Zoología Alfonso L. Herrera, Facultad de Ciencias, Universidad Nacional Autónoma de México; IBHED = Colección de Herpetología, Instituto de Biología, Universidad Nacional Autónoma de México.

Lab Protocols: Cloning

A clone of the mtDNA from an LS cytotype individual in the EMBL3 bacteriophage was available (Sites and Davis, 1989). A 2,400+ base pair (bp) *Eco*RI-*Bam*HI fragment from this phage clone was subcloned into the plasmid pUC12 using *Escherichia coli* (DH5-a) and designated pSgmt8 (Sites and Davis, 1989).

Sequencing Protocol

Plasmid DNA was isolated from overnight cultures of pSgmt8 in LB medium, by a modified version of the alkaline lysis plasmid mini-prep protocol (Kraft et al., 1988). Crude plasmid DNA preparations were treated with 2 μl of RNase A for 30 min at 37°C and then extracted once with PCI and once with chloroform and precipitated by the addition of 2.5 volumes of EtOH. Plasmid DNA was denatured prior to sequencing using 2 μl of 2 N NaOH as outlined by Kraft et al. (1988). Sequencing reactions were set up following the Sequenase protocol using the Sequenase enzyme version 2.0 (Tabor and Richardson, 1987; U.S. Biochemical Corp., 1987) and exposed to radiological film (Kodak Diagnostic Film SB 100, Rochester, NY) to visualize sequencing ladders.

Initial sequencing efforts used the Universal M13 primers (reverse 5'-TTC ACA CAG GAA A-3' and −40 5'-GTT TTC CCA GTC ACG AC-3' primers, both at concentrations of 10 ng/μl) and generated 250 bp of sequence at each end of the pSgmt8 clone. To complete sequencing both strands of the 2,400+ bp of the clone, we designed 33 internal primers (Table 2, Fig. 2) based on the sequence obtained for this LS clone and the bovine mtDNA sequence from GenBank. The pSgmt8 insert ran from the last 66 bp of the cytochrome oxidase III (COIII) gene through the leucine transfer RNA (LeutRNA). This fragment includes three different coding genes, ND3, ND4L, and ND4, and five transfer RNAs (tRNAs), Gly, Arg, His, Ser, and Leu (Fig. 2).

After collecting the complete sequence for the LS clone (2,479 bases), the same fragment was cloned and sequenced for the eight cytotypes, *S. g. grammicus*, and both outgroup taxa. Because pronounced intraspecific variation in mtDNA divergence may bias phylogenetic inference when sampling is restricted (Smouse et al., 1991), one representative of each cytotype was characterized to be "typical" of its own race, based on the more extensive restriction site mapping study by Sites and Davis (1989). A combination of two *S. grammicus*-specific primers, PIEco an PIIEco, that flank the entire target fragment was used to amplify genomic DNA, using the polymerase

TABLE 2. List of the mtDNA sequencing and PCR primers designed for *Sceloporus grammicus* cytotypes and outgroup taxa. Primers are listed from left to right from the 5′ to the 3′ ends. The sequence corresponds to the heavy strand of mtDNA. The order of the primers is according to their relative position along the mtDNA molecule, going from the cytochrome oxidase III (COIII) gene to the leucine tRNA (Fig. 2). Reference positions of the primers follow the bovine sequence (Anderson et al., 1982); parentheses identify heterologous primers.

Primer name	Reference positions	Sequence
PI-Eco	9688-9703	GGG AAT TCG ATA CTG ACA CTT CGT TGA CGT
PI′	9761-9781	CGA ACT AGT ACA GCT GAC TTC C
New Gly	9763-9782	ATA AGT ACA ATG (AC) (CT)T TCC A
Nap1	9907-9929	ACA GAA AAA CTA TCC CCA TAC GA
ND3 #2	9925-9944	TAC GAA TGT GGT TTT GA(CT) CC
ND3 Rev	9944-9925	GGG TCA AAT CCA CAT TC(AG) TA
NapRev	10011-9991	TTT GTC TTC TT(CT) ATT TTA ACG
ND3	10018-10049	GAA ATT GCC CTC CTT CTT CCA CTC CCA TGA GC
Arg	10136-10155	CCC AAA GGG GAC TAG AAT G
ND3 #3	10144-10163	GGA TTA GAA TGA GC(AC) GAA TA
Nap3	10150-10167	GAA TGA GCA GAA TTA AAC GT
ND4LNew	10309-10330	ACC TAA TAT CCG CCC TA(CT) TAT
ND4L	10323-10345	CTA CTA TGC TTT GAA GG(AT) ATA AT
Pollito	10502-10482	GTG GTC GTT ACC GTG AGT GCG
Pork	10756-10737	TAT TAG ATG AAG GAG TCA GC
Herp Term	10819-10798	GAT TAA GAA GGT TCG TT(TG) TCG
Gram C	10957-10937	TTG TCG TTC TGC TTG ATT CCC
Home Stretch	10966-10949	GTT CCA GCG GTT A(GA)T CGT TC
Home Stretch Rev	10949-10970	GAA CGA CTA ACC GCA GG(AG) ACA T
Bis	11114-11136	TGG GCC GCC TGC CTA CT(AT) GCC TT
ND4GapRev	11196-11177	GCT TCT ACA TGA GCT TT(AT) GG
Nap2	11200-11180	TGG AGC TTC TAC GTG (GA)GC TTT
ND4	11165-11196	CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC
Gram B	11268-11249	GTA ATT CGT ATA ATA CCG TA
ND4 #2	11358-11379	TAC GAC AAA CAG ACC TAA AAT C
ND4 Rev2	11384-11363	TTA ATG ATT TTA GAT CTG TTT G
ccND4	11536-11516	TCG TTC GTA GTT (AT)GT GTT TGC
Gram A	11604-11582	CAT CAG GTG GCT ATT AGT GGA A
ND4 Rev	11902-11885	TAT TAG GAG ATG TTC TCG
His	12002-11984	CAC TGC CTA ATG TTT TTG T
His3	12009-11991	TTA GAA TCA CAA TCT AAT
Leu	12086-12111	CAT TAC TTT TAC TTG GAT TTG CAC CA
PIIEco	12181-12162	GGG AAT TCG CTA CTT TTA CTT GGA GTT GCA

chain reaction (PCR; Saiki et al., 1985, 1988). Products were cloned using the lambda ZapII vector from Stratagene Cloning Systems. After all the target DNAs were subcloned into pBluescript, they were sequenced following methods of Sanger et al. (1977) with the 33 *S. grammicus*-specific primers (Arévalo, 1992).

Phylogenetic and Statistical Analysis

DNA sequences were input into the MacVector program (IBI-Kodak, version 3.5, 1991) and aligned against the bovine mtDNA sequence (Anderson et al., 1982) with the Clustal program (Higgins and Sharp, 1989), which allows a multiple sequence alignment and provides a similarity matrix for each possible pairwise comparison of sequences (Wilbur and Lipman, 1983). For each protein-coding gene, distance estimates (Kimura, 1980) were calculated and plotted on symmetrical dissimilarity matrices using the REAP software package (version 4.0; McElroy et al., 1991). The transition/transversion bias was checked for the entire fragment among the ingroup taxa and with both outgroups pooled together.

For phylogenetic analyses, each base position was treated as an unordered character with four alternative states. Ancestral and derived character states were deter-

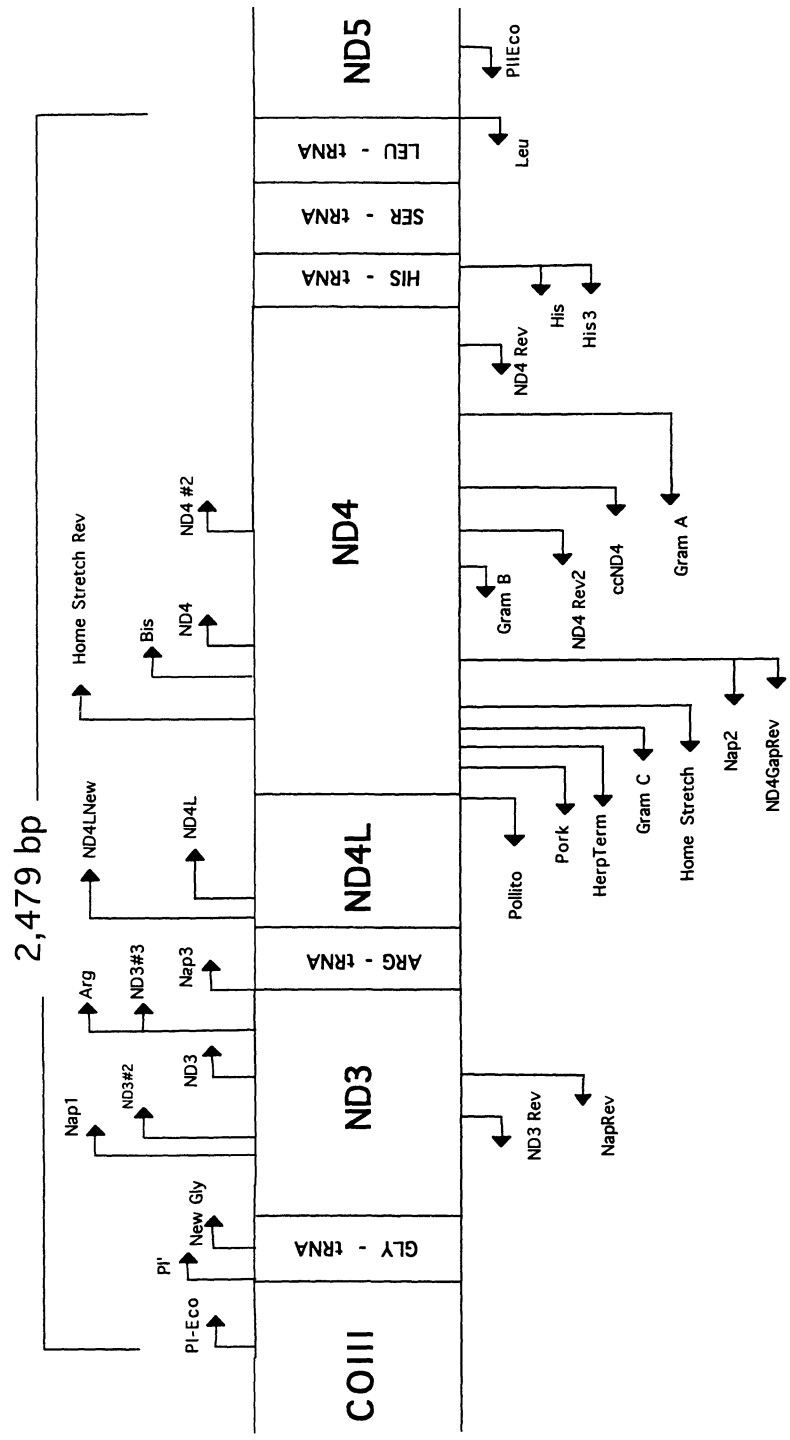

FIGURE 2. The DNA fragment of 2,479 bp sequenced for the study of *Sceloporus grammicus* chromosome races, showing approximate annealing positions of oligonucleotide primers. All arrows indicate the 5′ to 3′ direction of the primers, and their sequences are summarized in Table 2.

mined using the method of outgroup comparison (Watrous and Wheeler, 1981; Farris, 1982; Maddison et al., 1984). We originally chose *Sceloporus grammicus grammicus* as one possible outgroup because (1) it belongs to the *S. grammicus* complex and is characterized by the presumed $2n = 32$ ancestral karyotype and (2) it is confined to the Sierra Madre del Sur and is physically isolated from all *S. grammicus* populations on the central plateau area of Mexico. However, preliminary comparisons based on the first mtDNA sequences obtained in this study showed that *S. grammicus grammicus* was extremely similar to some of the ingroup taxa and raised the possibility that the *S. grammicus* cytotypes under study might be paraphyletic with respect to *S. g. grammicus*. We therefore included *S. g. grammicus* as an ingroup taxon and used *S. poinsetti* (from the *S. torquatus* group) and *S. olivaceus* (from the *S. horridus* group) as tentative first and second outgroups, respectively (Sites et al., 1992: fig. 26).

PAUP software (version 3.0s; Swofford, 1992) was used for the phylogenetic analysis. A distribution of tree lengths was generated for the entire fragment, and the test proposed by Hillis (1991) was used to distinguish phylogenetic signal from random noise (see also Huelsenbeck, 1991; Hillis and Huelsenbeck, 1992). Minimum-length trees were determined by rooting alternatively to either one of the two outgroups (*S. poinsetti* and *S. olivaceus*), as suggested by Donoghue and Cantino (1984) when relationships among outgroups are uncertain. Strict consensus trees were produced each time PAUP provided two or more equally parsimonious trees. We also used the bootstrap option of PAUP with 1,000 replications in the preliminary analyses as a compromise resampling analysis based on the size of the data matrix and number of analyses versus the optimal 2,000 replications recommended by Hedges (1992). Bootstrap resampling was carried out for the entire fragment and then for each of the protein genes under a variety of weighting options. Branch-and-bound searches were performed for all combinations of analyses for the entire fragment of 2,479 bp (including both protein-coding genes and tRNAs), for the protein-coding genes individually (ND3, ND4L, and ND4), for all the protein-coding genes together (2,087 bp), and for all the tRNAs together (351 bp). For the entire fragment, an exhaustive search was performed using either one of the outgroups, *S. olivaceus* and *S. poinsetti*. However, because the statistics were the same as for the branch-and-bound search, the individual gene analyses were performed only using the branch-and-bound methodology.

In addition to the first analysis based on equal character weighting (transitions = transversions, all codon positions retained), four weighting methods were tested: (1) transitions/transversions (weighted in favor of transversions, 1:2), (2) transversions only, (3) elimination of the third base positions (these three options provided a first-order test of positional heterogeneity common in most protein sequences [Li et al., 1985a, 1985b; Felsenstein, 1988]), and (4) all 12 possible substitution types ($A \rightarrow C$, $C \rightarrow A$, $A \rightarrow G$, $G \rightarrow A$, $A \rightarrow T$, $T \rightarrow A$, $C \rightarrow G$, $G \rightarrow C$, $C \rightarrow T$, $T \rightarrow C$, $G \rightarrow T$, and $T \rightarrow G$) proportional to their observed (inferred) frequencies, as estimated by MacClade 3.0 (Maddison and Maddison, 1992). Details of this 12-parameter weighting method are given below, and results were compared across all methods for congruence of tree topologies. The single "best" hypothesis was then selected on the basis of the structure of the model underlying the weighting method and the bootstrap values obtained relative to all alternatives. The strength of the hypothesis was evaluated by comparing its length to the number of steps in alternative trees constrained to match the general topologies of those presented in Figure 1, and the difference was tested using the winning-sites test of Prager and Wilson (1988).

RESULTS

Figure 3 shows the entire sequence of the light strand for the 10 ingroup taxa, including the eight known cytotypes of the *S. grammicus* complex, the original pSgmt8 clone upon which the primer se-

quences were based, *S. grammicus grammicus*, and both outgroup taxa. Numbers correspond to the bovine sequence (Anderson et al., 1982).

Sequence Variation: Protein-coding Genes

Table 3 summarizes the variation, first across all ingroup taxa and then for both outgroups, by codon position (first, second, and third base position) and substitution type (transition/transversion) for all protein-coding genes. For all protein bases (2,087 positions, excluding primers), a total of 853 varied in one or more of the 10 ingroup taxa (45.66%). Of the total variable positions, 659 (69.15%) corresponded to third, 81 (8.50%) to second, and 213 (22.35%) to first base positions. Of the variable third positions, 463 (70.26%) were transitions and 196 (29.74%) were transversions; of the variable second positions, 68 (83.95%) were transitions and 13 (16.05%) were transversions; and of the variable first positions, 166 (77.93%) were transitions and 47 were (22.07%) transversions.

Tables 4 and 5 show pairwise comparisons of Kimura's (1980) genetic distances, for the individual genes and for the entire sequence, for all the taxa analyzed. The Kimura distances for the ND3 gene (Table 4) ranged from 0.01 (pSgmt8 vs. F6, FM1 vs. FM3) to 0.26 (FM1 vs. *S. poinsetti*). For the ND4L gene (Table 4), the Kimura distances ranged from 0.00 (pSgmt8 vs. F6) to 0.23 (pSgmt8 vs. *S. olivaceus*). Values for the ND4 gene (Table 5) ranged from 0.00 (pSgmt8 vs. F6) to 0.23 (pSgmt8 vs. *S. olivaceus*).

Figure 4 shows the amino acid sequence translated from each of the complete protein genes studied aligned against the bovine reference sequence. In addition, we have included data for *Xenopus* (Roe et al., 1985) and chicken (Desjardins and Morais, 1990), because some coding regions in the bovine sequence were quite different from those of any of the lizards. All three genes contain regions with very conserved amino acid sequences shared both within the ingroup and between the ingroup and outgroups. However, other regions were more variable, and the amino acids were only shared within the ingroup taxa. Thomas and Beckenbach (1989) suggested that for mtDNA protein-coding genes there is a reduced sequence divergence at the junction of different genes, probably due to functional constraints. This pattern was not observed in the three protein sequences analyzed in the present study; regions of amino acid sequence similarity were more pronounced in the interior regions of the gene. For example, the central region of the ND3 gene (bases 9,006–9,052 for the bovine mtDNA sequence) was virtually identically across all taxa, with only 11 substitutions, whereas on both extremes of the gene many more differences were found.

Sequence Variation: tRNA Genes

A total of 344 bp (14% of the total sequence) comprised the five tRNAs sequenced in this study. Of these 344 bp, 292 positions (84.88%) were identical across all ingroup taxa, and 52 (15.12%) were variable. Of these substitutions, 36 (69.23%) were transitions and 16 (30.77%) were transversions. Forty-five sites (12.82%) differed between the ingroup taxa and *S. olivaceus*, and 57 (16.24%) differed between the ingroup taxa and *S. poinsetti*. Of these differences, 16 and 17 were unique differences for *S. olivaceus* and *S. poinsetti*, respectively.

Preliminary Phylogenetic Analyses

Exhaustive searches for the entire aligned fragment recovered single most-parsimonious trees when rooted with either outgroup (Fig. 5), and both appeared to contain phylogenetic signal as measured by the skewness statistic g_1: -0.713 and -0.715 (Hillis, 1991). (We do not interpret the g_1 values as quantitative measures of signal [contra the claim made by Källersjö et al., 1992, for the original intent of its use] but only as an indication that signal is detected relative to random variation distributed among taxa independent of phylogenetic history.) The tree obtained by rooting with *S. poinsetti* was slightly shorter than its alternative (1,325 vs. 1,342 steps), but both solutions had similar consistency indexes (CIs; 0.529 vs. 0.532).

Branch-and-bound searches for individual protein genes revealed congruence be-

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                9681
                1          COIII
Bovine          TGA TAC TGA CAT TTC GTA GAC GTA GTC TGA CTT TTC CTC TAT GTT TCT ATC TAT TGA TGA GGC TCC TA
pSgmt8          ... ... ... ..C ... ..T ... ..T ..T ... ..A ... ..G ..C ..A ..A ..T ... ... ... ..A ..T .G
LS              x.. ... ... ..C ... ..T ... ..T ..T ... ..A ... ..A ..C ..G ..A ..T ... ... ... ..T ..G .G
SGG             x.. ... ... ..C ... ..T ... ..T ..T ... ..A ... ..G ..C ..A ..A ..T ..C ... ... ..A ..T .G
F5              x.. ... ... ..C ... ..T ... ..T ..T ... ..A ... T.A ..C ..A ..C ..T ... ... ..G ... ..T .G
F6              x.. ... ... ..C ... ..T ... ..T ..T ... ..A ... ..G ..C ..A ..A ..T ... ... ... ..A ..T .G
FM2             ... ... ... ..C ... ..T ... ..T ..T ... ..A ... ..A ... ..A ..A ..T ..C ... ... ... ..T .G
HS              x.. ... ... ..C ... ..T ... ..T ..T ... T.A ... ..A ... ..A ..A ... ..C ... ... ... ..T .G
F5+6            x.. ... ... ..C ... ..T ... ..T ..T ... ..A ... ..A ... ..A ..A ..T ..C ... ... ... ..T .G
FM3             x.. ... ... ..C ... ..T ... ..C ..T ... ..A ... T.A ..C ..A ..C ..T ..C ... ... ... ..T .G
FM1             x.. ... ... ..C ... ..T ... ..C ..T ... ..A ... T.A ..C ..A ..C ..T ..C ... ... ... ..T .G
SO              x.. ... ... ..C ... ..T ... ..T ..T ... ..G ... T.G ..C ..A ..A ... ..C ... ... ... ..T .G
SP              x.. ... ... ..C ... ..T ... ... ..A ... ..A ... ..A ..C ..C ..A ... ..C ... ... ... ..A ..T .G

                ** GLY-tRNA                                                                   * ND3
Bovine          TTCTTTTAGTATTAACTAGTACAGCTGACTTCCAATCAGCTAGTTTCGGTCTAGTCCGAAAAAGAAT A ATA AAT TTA ATA CTA
pSgmt8          ......................AG.........C...CTA...C.TAA.T..ACTTA.G......C . ..G ... ... .C. AC.
LS              ..............T.......AG.........C...CTA..CC.TAA.AA.A.TTA.G......C . ..G ... ... .C. AC.
SGG             ......C...............AG.........C...CTA...C.TAA.T..ACTTA.G......C . ..G ... ... .C. AC.
F5              ..............T.......AG.........C...CTA..CC.TAA.AA.ACTTA.G......C . ..G ... ... .C. AC.
F6              ......................AG.........C...CTA...C.TAA.TT.ACTTA.G......C . ..G ... ... .C. AC.
FM2             ............C.GT.......AG.........C...CTA..CC.TAA.AA.ACTTA.G......C . ..G ... ... .C. AC.
HS              ..............C......AG.........C...CTA..CC.TAA.AG.ACTTA.G......C . ..G ..C ... .C. AC.
F5+6            ..............T.......AG.........C...CTA..CC.TAA.AA.ACTTA.G......C . ..G ... ... .C. AC.
FM3             ..............TC......AG.........C...CTA..CC.TAA.AA.ACTTA.G......C . ..G ... C.. .C. AC.
FM1             ..............TC......AG.........C...CTA..CC.TAA.AA.ACTTA.G......C . ..G ... C.. .C. AC.
SO              ............C..C......AG.........C...CTA..CC.TAA.AA.ACTTA.G......C . ..G ..C ... .C. AC.
SP              ............-..CC......AG.........C...CTA...C.TAA.AA.ACTTA.G......C . ..G ..C ... .C. AC.

Bovine          GCC CTC CTG ACC AAT TTT ACA CTA GCC ACC CTA CTC GTC ATC ATC GCA TTC TGA CTT CCC CAA CTA
pSgmt8          ATA ..A A.T G.T TCA C.. .T. G.T T.A T.A ... ... A.. ..A ..T AGC ..T ... ..C ... ... ...
LS              ATA ..A A.T G.T TCA C.. .T. G.T T.A T.A ... ... A.. ..A ..T AGC ..T ... ..C ... ... ...
SGG             ATA ..A A.T G.T TCA C.. .T. G.T T.A T.A ... ... A.. ..A ..T AGC ..T ... ..C ... ... ...
F5              ATA ..A A.T TTT TCA C.. .T. G.T T.A TTA ... ..T A.. ..A ..T GGC ..T ... T.A ..A ... .CC
F6              ATA ..A A.T G.T TCA C.. .T. G.T T.A T.A ... ... A.. ..A ..T AGC ..T ... ..C ... ... ...
FM2             ATA T.A A.C TTT TCA C.. .T. A.T T.A TTA ... ..T A.T C.A G.T AGC ..T ... ..A ..A ... ..C
HS              ATA ..A A.T TTT TCA C.A .T. G.C T.A CTA ... ..T A.. C.A ..T AGC ..T ... T.A ... ... ...
F5+6            ATA ..A A.T TTT TCA C.. .T. A.T T.A TTA ... ..T A.T T.A C.T AGC ..T ... T.A ... ... ..C
FM3             ATA ..A A.C TT. TCA C.. .T. A.T T.A TTG ... ..T A.T C.A G.T ..C ..T ... T.A ..A ... .CC
FM1             ATA ..A A.C TT. TCA C.. .T. A.T T.A TTG ... ..T A.T C.A G.T ..C ..T ... T.A ..A ... .CC
SO              ATA ..T ..C .T. TCC C.. .TC A.C T.A G.. ..G ..T A.. C.T ..T AGC ... ... ..A ..A ... ...
SP              ATA ..A ..A .TT TCA C.. .T. A.C T.. G.. ... ..T A.. C.A ..T AGT ... ... ..A ..A ... T.C


Bovine          AAT GTA TAC TCT GAG AAA ACA AGC CCA TAC GAA TGT GGA TTT GAC CCC ATA GGA TCA GCC CGC CTT
pSgmt8          T.C CCC G.T A.A ..A ... CT. TC. ... ... ... ... ..G ... ... ..A C.T ... AAC ... ..A ...
LS              T.C CCC G.T A.A ..A ... CT. TC. ... ... ... ... ..G ... ... ..A C.T ... AAC ... ..A ...
SGG             TTA .CC CCG ATA C.. ... CT. TC. ... ... ... ... ..G ... ... ... C.T ... AAC ... ..A ...
F5              TGC CCT G.T A.A ..A ... CT. TCT ... ... ... ..C ... ... ..T ..A C.T ... AAC ..T ..A ...
F6              T.C CCC G.T A.A ..A ... CT. TC. ... ... ... ... ..G ... ... ..A C.T ... AAC ... ..A ...
FM2             T.C CC. G.. A.A ..A ... TTG TCT ... ... ... ... ... ... ... ... C.T ... AAC ..T ..A ...
HS              T.C CC. G.. CTA ..A ... CT. TC. ... ... ... ..C ... ... ... ... C.T ... AGC ... ..A ...
F5+6            T.C CC. G.. A.A ..A ... CT. TC. ... ... ..G ... ... ... ..T ..T C.T ... AAC ..T ..A ...
FM3             T.C CCT G.. A.A ..A ... CT. TC. ... ... ... ..C ..G ... ..T ..A C.T ... AAC ..T ..A ...
FM1             T.C CCT G.. A.A ..A ... CT. TC. ... ... ... ..C ..G ... ..T ..A C.T ... AAC ..T ..A ...
SO              T.. CC. G.. A.A ..A ... CT. TCT ... ... ... ..C ... ..C ..T ... C.T ... AAC ... ..A ...
SP              C.C CC. G.. A.A ..A ..G CT. TC. ... ... ... ... ... ... ... ..A C.C ... AGC ... ..A ..C

Bovine          CCC TTC TCT ATA AAA TTC TTT CTG GTA GCC ATC ACA TTC CTC TTA TTT GAC CTA GAA ATT GCA CTC
pSgmt8          ..A ... ..A C.. CG. ..T ..C T.A ... ..A ..T TT. ..T ... C.G ... ... ..T ... ... ..C ..T
LS              ..A ... ..A C.. CG. ..T ..C T.A ... ..A ..T TT. ..T ..T C.. ... ... ..T ... ... ..C ..A
SGG             ..A ... ..A C.. CG. ..T ..C T.A ... ..A ..T TT. ..T ... C.G ... ... ..T ... ... ..C ..T
F5              ..A ..T ..A C.T CG. ... ..C ..A ..G ..A ..T TT. ..T ..T C.. ... ... ..T ... ... ..C ..A
F6              ..A ... ..A C.. CG. ..T ..C T.A ... ..A ..T TT. ..T ... C.G ... ... ..T ... ... ..C ..T
FM2             ..A ..T ..A C.T CG. ... ..C T.A ..G ..A ..T TT. ..T ..T C.. ... ... ..T ... ... ..C ..G
HS              ..A ... ..A C.. CG. ..T ..C ..A ... ..A ..T TT. ..T ..T C.G ... ... ..T ... ... ..C ..A
F5+6            ..A ..T ..A C.T CG. ... ..C T.A ... ..A ..T TT. ..T ..T ... ... ... ..T ... ... ..C ..A
FM3             ..A ..T ..G C.T CG. ... ..C ..A ..G ..A ..T TT. ..T ... C.. ... ..T ..T ... ... ..C T.A
FM1             ..A ..T ..G C.T CG. ... ..C ..A ..G ..A ..T TT. ..T ..T C.. ... ..T ..T ... ... ..C T.A
SO              T.A ..T ..A C.. CG. ... ..C ..A ... ..T ..T CT. ..T ..T ... ... ... ..T ... ..C ..C ..A
SP              ..A ... ..A C.. CG. ..T ..C ..A ... ..A ... CTG ... ... C.. ... ... ..T ... ..C ..C ..A
```

FIGURE 3. The mtDNA sequences for all *Sceloporus* ingroup and outgroup taxa examined in this study (including *S. grammicus grammicus* and the pSgmt8 clone) aligned against the light strand of the bovine mitochondrial sequence (Anderson et al., 1982). All sequences are deposited in GenBank under accession nos. L32578-L32587 and L33838.

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Bovine    CTC CTA CCA CTG CCA TGA GCC TCA CAA ACA GCA AAT CTA AAC ACA ATG CTT ACC ATA GCC CTC TTC
pSgmt8    T.A ... ... ..A ..T ... ... ... A.. CT. AA. ..A TC. .CA CT. .CC A.A ..T C.T .TA AC. A.T
LS        ..A T.. ... ..A ... ..G ... ... A.. CT. AA. ... .C. .CA CT. .CT A.A ..A C.T .TA ACT ACT
SGG       T.A ... ... ..A ..T ... ... ... A.C CT. AA. ... .C. .CA CT. .CC A.A ..T C.T .TA AC. A.T
F5        ..A T.. ... ..A ..T ... ... ... A.C CT. AA. ... .C. .CA CT. .CT A.A .TA T.T .TA AC. ACT
F6        T.A ... ... ..A ..T ... ... ... A.C CT. AA. ... .C. .CA CT. .CC A.A ..T C.T .TA AC. A.T
FM2       T.A T.. ..C ..G ..T ..G ... ... A.C CT. AA. ... .C. .CA TT. .CT A.A G.A T.T .TA AC. ACT
HS        T.A T.. ..G ..A ..C ... ... ..T A.C CT. .A. ..C .C. .CA TT. TCA ACA ..A C.T .TA AC. AC.
F5+6      T.A T.. ..C ... ... ..G ... ... A.C CT. AA. ... .C. .CA TT. .CT T.A G.A T.T .TA ACT AC.
FM3       ..A T.. ... ... ..T ... ... ... A.C CT. AA. ..C .C. .CA TT. .CT A.A .TA T.T .TA ACT ACT
FM1       ..A T.. ... ... ..T ... ... ... A.C CT. AA. ..C .C. .CA TT. .CT A.C CTA T.T .TA ACT ACT
SO        ..A ... ... ..A ..G ... ... ... A.C CT. AA. ..C .C. .TA GTG .CC A.A .TA C.C ATA AC. A.T
SP        T.A ... ... ..A ... ... ..G ... A.C CT. AA. ... .C. .CA GTG .CT A.A .TT C.T A.A ACT GCT

Bovine    CTA ATT ATC CTC CTA GCT GTA AGC CTA GCC TAT GAG TGA ACT CAA AAA GGA CTA GAA TGA ACC GAA
pSgmt8    A.T C.C C.A ..A ... A.. C.T G.. ... AT. ... ..A ... ..C ... GGG ... ... ... ... G.A ...
LS        A.T C.C C.. ..A ... A.. A.T G.T T.. ATT ... ..A ... G.C ... GGG ..T T.. ... ..G G.A ...
SGG       A.T C.C C.A ..A ... A.. A.T G.. ... AT. ... ..A ... G.C ... GGG ... ... ... ... G.A ...
F5        A.T C.C C.A ..A T.. A.. A.t G.. ... ATT ... ... ... T.C ... GGT ..G T.. ... ... G.A ...
F6        A.T C.C C.A ..A ... A.. C.T G.. ... AT. ... ..A ... ..C ... GGG ... ... ... ... G.A ...
FM2       A.T C.A ..T ..A ... AT. A.. G.. ... ATT ..C ..A ... ..C ... GG. ... ... ... ... G.A ...
HS        A.C C.A C.T ..A ... A.. A.T G.G T.. AT. ... ... ..G ... ... GG. ... T.. .CC ... G.A ...
F5+6      A.T C.A ..A ..A ... AT. A.C G.. ... ATT ..C ..A ... ... ... GG. ... T.. .CC ... G.A ...
FM3       A.T C.. C.A ..A T.. A.C A.T G.. ... ATT ... ... ... T.C ... GGT ..G T.. ... ... G.A ...
FM1       A.T C.. C.A ..A T.. A.C A.T G.. ... ATT ... ... ... T.C ... GGT ..G T.. ... ... G.A ...
SO        A.C C.G C.A ... ... A.A C.T G.. ... AT. ... ..A ... T.C ... GGG ..C ... ..G ..G G.A ...
SP        A.C C.C C.. T.A ..T A.A C.T G.A T.. ATT ... ... ... ..C ... GG. ..C ... ... ... G.A ...

              ** ARG-tRNA                                                                  * ND4L
Bovine    TAT -- GGTACTTAGTTTAAAATAAAATAAATGATTTCGACTCATTAGATTATGATTTAATTCATAATTACC-A A ATG TCT
pSgmt8    .TA AA C..---..ATC....-C..G...GT.............AC.T..CTA.GA.AT..C.C..GACGTTT. C ... .TA
LS        .TA AA C..---..ATC....-C..G..GGT.............AC.T..CTA.GA.AT..C.C..GACGTTT. C ... .TA
SGG       .TA AA C..---..ATC....-C..G...GT.............AC.T..CTA.GA.AT..C.C..GACGTTT. C ... .TA
F5        .TA AA C..---..ATC...G-C..G...GT.............AC.T.CCTA.GACATTCC.C..GACGTTT. T ... .TA
F6        .TA AA C..---..ATC....-C..G...GT.............AC.T..CTA.GA.AT..C.C..GACGTTT. C ... .TA
FM2       .TA AA C..---..ATC....-C..G..GGT.............AC.T..CTA.G-A.GT.C.C..GACGTTT. C ... .TA
HS        .TA AA C..---..ATC....-C..G...GT.............ACCT..CTA.G-ACGCGC.C..GACGTTT. C ... .TA
F5+6      .TA AA C..---.GATC....-C..G...GT.............AC.T..CTA.G-AC.TCC.C..GACGTTT. C ... CTA
FM3       .TA AA C..---..ATC....-C..G...GT.............ACCC..CTA.G-AC.T-C.C..GACGTTT. T ... .TA
FM1       .TA AA C..---..AtC....-C..G...GT.............ACCc..CTA.G-AC.T.C.C..GACGTTT. T ... .TA
SO        .TA GG C..---.G.TC....-C..G...GT.............AC.G..CCA..A.GCTCG.C.GGACG..T. C ... .TA
SP        .TA GA C..---....A....-C......GT.............AC.G..CTA.GA..T.CC.C.TGACGT.T. T ... CTA

Bovine    ATA GTA TAC ATA AAC ATT ATA ATA GCA TTC ACA GTA TCT CTT GTA GGA CTA CTA ATA TAC CGA
pSgmt8    CC. A.. C.. T.T .CA C.A .AT TC. A.. ..T .TT C.T AGC A.. A.. ..T A.. TC. ..T C.. ...
LS        CC. A.. C.. T.T .CA C.A .AT TC. A.. ..T .TT C.T AGC A.. A.. ..T A.. TC. ..T C.. ...
SGG       CC. A.. C.. T.T .TA C.A .AC TC. A.. ..T .TT C.C AGC A.C A.. ..C A.. TCT T.. C.T ...
F5        CCC A.G C.T T.T .CA C.A .AC TC. A.. ..T .TC C.C AGC A.C A.. ... ... TC. ... C.. ...
F6        CC. A.. C.. T.T .CA C.A .AT TC. A.. ..T .TT C.T AGC A.. A.. ..T A.. TC. ..T C.. ...
FM2       CCT A.. C.. T.T .CA C.A .AC TC. A.. ..T .TC C.C AGC A.. A.G ... ... TC. ..G C.. ...
HS        CCT A.. C.T T.T .CA C.A .AC TC. A.. ..T .TT C.T AGC A.. A.. ..C T.. TC. ... C.. ...
F5+6      CCT ... C.C T.T .TA C.A .AC TC. A.G ..T .TC C.C AGC A.. A.G ... T.. TC. ..G C.. ...
FM3       CCC A.. C.T T.C .CA C.A .AC TC. A.. ..T .TC C.. AGC A.. A.. ... ... TC. ..G C.. ...
FM1       CCC A.. C.T T.C .CA C.A .AC TCT A.. ..T .TC C.T AGC A.. A.. ... ... TC. ..G C.. ...
SO        CC. A.. C.T T.T .CA C.A .AC TC. A.. ... .TC C.. AGC A.C C.G ..C ... TC. ... C.. ...
SP        CC. A.. C.T T.C .C. ..A .AC TC. A.. ... .TC C.T AGC A.. A.. ..C T.. TC. ... C.. ...

Bovine    TCC CAC CTA ATA TCC TCC CTT CTA TGC TTA GAA GGA ATA ATG CTA TCC CTA TTC GTT ATA GCA
pSgmt8    ATA ..T ... ..G ... G.. ..A ... ..T A.T ..G ... ... ..A T.. G.. ... ... A.. ..T ATT
LS        ATA ... ... ..G ... G.. ..A ... ..T A.T ... ..C ... ..A T.. G.. ... ... A.. ..T ATT
SGG       ATA ... ... ... ... G.. ..A ... ..T A.T ..G ... ... ..A T.. G.. ... ... A.. ..T ATT
F5        ATA ... ... ... ... G.. ..A T.. ..T A.T ..G ..T ... ..A ... G.. ... ..T A.. ..T ATT
F6        ATA ..T ... ..G ... G.. ..A ... ..T A.T ..G ... ... ..A T.. G.. ... ... A.. ..T ATT
FM2       ATA ... ... ... TCA G.. T.A T.. ..T A.T ..G ..C ... ..A ... G.. ... ..T A.. ..T ATT
HS        ATA ... ... ..G ... G.. ..A ... ..T A.T ... ..C ..G ... T.. G.. ... ..T A.. ..T ATT
F5+6      ATA ... ... ... TCA G.. ..A ... ..T A.T ... ..C ... ... ... G.. ... ..T A.. ..T ATT
FM3       ATA ... ... ... ... G.. ..A T.G ..T A.T ..G ..T ... ... ... GTT ... ..T A.. ..T ATT
FM1       ATA ... ... ... ... G.. ..A T.G ..T A.T ..G ..T ... ... ... GTT ... ..T A.. ..T ATT
SO        A.A ..T ... ... ... G.. ..A T.. ... A.T ... ..C ... ... ... T.. G.. T.. ..T A.. ..T A.C
SP        A.A ... ..T ... ..G G.. ..A T.. ... A.T ... ..T ... ... ... ... G.. ..C ... A.C ..T A..
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FIGURE 3. Continued.

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Bovine   GCC CTA ACA ATC CTC AAC TCA CAT TTT ACA TTA GCT AGC ATA ATA CCT ATT ATC CTA CTA GTC
pSgmt8   A.A AC. TTC T.T TCA .CT AAC A.C C.A CA. ACC ATG GCA CCC .CC ..A ... ..A ..T ..G .CT
LS       A.A AC. TTC T.T TCA .CT AAC A.C C.C CA. ACC ATA GCA CCC GCC ..A ... ..A ..T ..G .C.
SGG      A.A AC. TTC T.T TCA .CT AAT AGC C.A CA. ACC ATG GCA CCC .CC ..A ... ... ..T ..G .CT
F5       A.A AT. TTC T.T TCA .CT AAT A.. ..A CAG ACC ATG GC. CC. GCC ..A ... ..A ..C ... .CT
F6       A.A AC. TTC T.T TCA .CT AAC A.C C.A CA. ACC ATG GCA CCC .CC ..A ... ..A ..T ..G .CT
FM2      T.A AC. TTC T.T TCA .CT AAT A.. ..A CAG ACC ATG GC. CC. GCC ..A ... ..A ..T ..G .CT
HS       A.A AC. TTC T.T TCA .CA AAC A.C C.C CA. ACC ATA GCA CCC GCC ..A ... ..A ..T ..G .CT
F5+6     T.A AC. TTC T.T TCA .CT AAT A.. ..A CA. ACC ATG GC. CC. GCC ..A ... ..A ..C ..G .CT
FM3      A.A AC. TTC T.T TCA .CT AAT A.. ..A CA. ACC ATA GC. CC. GCC ..A ... ..A ..C ... .CT
FM1      A.A A.. TTC T.T TCA .CT AAT A.. ..A CA. ACC ATA GC. CC. GCC ..A ... ..A ..C ..G .CT
SO       ..A AC. TTC T.T TCA .CA AAT A.C A.. CA. ACC ATA GCA CC. .C. ..C ... ..G ... ... .CT
SP       A.A AC. TTT T.T TCA .CA AAC A.C C.C CA. ACC ATA GCA TC. GC. ..A ... ..A ... ... .CT

Bovine   TTC GCA GCC TGT GAA GCA GCC CTA GGT CTA TCT CTA CTA GTA ATA GTA TCA AAT ACA TAT GGT
pSgmt8   ..T T.. ... ... ... ... AG. ACT ... ... G.C T.. A.. ... GC. ACC ..C CG. ..C ..C ..C
LS       ... T.. ... ... ... ... AG. ACT ... ... G.C T.. A.. ... GC. ACC ..C CG. ..C ..C ..C
SGG      ... T.. ... ... ... ... AG. ACT ..C ... G.C T.. A.. ... GC. ACC ..C CGC ..T C.C ..C
F5       ... T.. ... ... ... ... AG. A.C ... ... G.C T.. A.. ... GC. ACT ..C CGC ..C C.C ..C
F6       ... T.. ... ... ... ... AG. ACT ... ... G.C T.. A.. ... GC. ACT ..C CGC ..C C.C ..C
FM2      ..C T.. ... ... ... ... AG. A.T ... ... G.C T.. A.. ... GC. ACT ..C CGC ..C C.C ..C
HS       ..C T.. ..T ... ... ... AG. ACC ..C T.. G.C T.. A.. ... GC. ACC ..T CGC ..T C.C ..C
F5+6     ..A T.. ... ..C ... ... AG. A.T ... ... G.C T.. A.. ... GC. ACC ..C CGC ..C C.C ..A
FM3      ... T.. ... ... ... ... AG. A.T ... ... G.C T.. A.. ... GC. ACT ..C CG. ..C ACA ..C
FM1      ... T.. ..T ... ... ... AG. A.T ... ... G.C T.. A.. ... GC. ACC ..C CGC ..C C.C ..A
SO       ..C T.. ... ..C ..G ... AGT G.C ..C ..G G.C ... A.. ..G GCG ACC ..G CGC ..T C.C ...
SP       ..C T.. ... ..C ... ... AG. A.T ..C ... G.C T.. ... ... GC. AC. ..C CGC ..T C.C ..C

                                                    ** ND4
Bovine   ACT GAT TAT GTA CAA AAC CTC AAC TTA CTC CA ATG CTA AAA TAC ATT ATT CCA ACA ATT ATA
pSgmt8   .AC ..C A.C C.T A.. ... ..A ... ... ..A .. ... T.. ... GTA ... T.A ..C ... C.A ...
LS       .AT ... A.C C.T A.. ... ..A ... ... ..A .. ... T.. ... AGT ... T.A ... ... C.A ...
SGG      .AC ..C G.C C.T A.. ... ..A ... ... ..A .. ... T.. ... GTA ... T.A ... ... C.A ...
F5       .AC ... A.C C.T A.. ... ... ... C.. T.A .. ... ... ... GTA ..C C.A ... ... C.A ..T
F6       .AC ..C A.C C.T A.. ... ..A ... ... ..A .. ... T.. ... GTA ... T.A ..C ... C.A ...
FM2      .AC ..C AGC C.T A.. ... ..A ... C.. ..G .. ... ... ... GTA ..C T.A ... ... C.A ...
HS       .AT ... A.C C.T A.. ... T.A .GT C.. ..G .. ... ... ... GTA ... T.A ..C ... T.A ...
F5+6     .AC ..C A.C C.T A.. ... ..A ... C.. ..G .. ... ... ... GTA ... T.A ... ... C.A ...
FM3      .AC ... A.C C.T A.. ... ..A ... C.. T.A .. ... ... ... GTA ..C T.A ... ... C.G ..T
FM1      .AC ..C A.C C.T A.. ... ..A ... C.. ..G .. ... ... ... GTA ... T.A ... ... C.A ...
SO       .AC ..C C.C C.T A.. ... ..A ... C.T ..A .. ... ... ... ATT ... C.A ... ... C.A ..G
SP       .G. ..C C.C C.T A.. ... ..A ... C.C T.A .. ... T.. ... ATA ... T.A ... ... C.A ...

Bovine   CTT ATA CCC CTA ACC TGG TTA TCA AAA AAT AAT ATA --- ATT TGG GTT AAC TCC ACA GCA CAC
pSgmt8   ..A GCC ..A ACT G.T ATA AC. A.. ... CCA TTA TAT ACA T.. A.C C.A TT. A.A ... TAC TCA
LS       T.A GCC ... ACT G.C AT. AC. A.. ... CCA ATA TAC ACT T.. AAC C.A TTT A.A .T. TAC TCA
SGG      ..A GCC ..A ACT G.T ATA AC. A.. ... CCA TTA TAT ACT T.. A.C T.A TT. A.A ... TAC TCA
F5       ..A GC. ..A ACT G.. AT. AC. A.. ... CCA CTA TAT ACA T.. AAC C.A TT. A.A .T. TAC TCA
F6       ..A GCC ..A ACT G.T ATA AC. A.. ... CCA TTA TAT ACA T.. A.C C.A TT. A.A ... TAC TCA
FM2      ..A GCG ..A ACT G.. AT. AC. A.. ... CCA CTA TAC ACA T.. AAT C.A TT. A.A ... TAC TCA
HS       T.A GCC ... ACT G.. AT. Ac. A.. ... CGC C.A TAT ACA C.. AAC C.A TTT A.A .T. TAC TCA
F5+6     ..A GCG ..A ACT G.. AT. AC. A.. ... CCA CTA TAT ACA T.. AAC C.A TTT A.A ... TAC TCA
FM3      ..A GC. ..A ACT G.. ATA AC. A.. ... CCA CCC TAT ACA T.. AAC C.A TT. A.A .T. TAC TCA
FM1      ..A GCG ..A ACT G.. AT. AC. A.. ... CCA CTA TAT ACA T.. AAC C.A TTT A.A .T. TAC TCA
SO       ..C GC. ..A ACC G.. ATA G.. A.. ... CCA .CA TAC ACA T.. A.C C.G TT. A.A .T. TAC TCA
SP       ... GC. ..A ACT G.. ATA A.. G.. ... CCA CCA TAC ACA T.. A.C A.A TT. A.A G.. TAC .CA

Bovine   AGC CTT CTA ATT AGC TTT ACA AGC CTC CTC CTC ATA AAC CAG TTT GGC GAC AAC AGC CTT AAT
pSgmt8   .C. GCC .T. GCA CTA A.. .GT CTA ACT TGA ..A .A. TCA .CA A.A AA. ATA G.A TCA ACA TT.
LS       .CT A.C .T. GCA CTA A.. .GT CTA ACT TGA ..A .A. TCA .CA A.A AA. ATA G.A TCA ACA TT.
SGG      .C. A.C .T. GCA TTA A.. .GC CTA ACT TGA ..A .A. TTA .CA A.A AA. ACA G.A CCC ACA TT.
F5       .CA A.C TT. GCA CTA A.. .GC CTA ACT TGA ..A .A. TCA TCA A.A AA. ACA G.A CCA ACA TT.
F6       .C. GCC .T. GCA CTA A.. .GT CTA ACT TGA ..A .A. TCA .CA A.A AA. ATA G.A TCA ACA TT.
FM2      .TA A.C .T. GCA CTA A.. .GC CTA ACT TGA T.A .A. TCA TCA A.A AAT ACA G.A CCT ACA TT.
HS       .C. A.C .T. GCA TTA A.. .GC CTA ACT TGA ..A .A. TTA .CA A.A AA. ACA G.A CCT ACA TT.
F5+6     .TA A.C .T. GCA CTA A.. .GC CTA ACT TGG T.A .A. TCA TCA A.A AAT ACA G.A CCA ACA TT.
FM3      .CA A.C TT. GCA CTA A.. .GC CTA ACT TGA ..A .A. TCA TCA A.A AA. ACA G.A CCA ACA TT.
FM1      .TA A.C .T. GCA CTA A.. .GC CTA ACT TGA ..A .A. TCA TCA A.A AA. ACA G.A CCA ACA TT.
SO       .C. ACC TTG GCA TTA A.C .GC CT. ACA TGA ..A .A. ACA TCA A.A AA. ACA G.A CCG ACA TTC
SP       .C. A.. .T. GCA CTA A.. .GC CTA ACT TGG ..A .A. ACA TCA A.A AA. ACA G.A CCT ACA TTC
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FIGURE 3. Continued.

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Bovine    TTT TCA CTA CTA TTT TTC TCC GAC TCC CTA TCC ACT CCA CTA CTA ATT TTA ACC ATA TGG CTC
pSgmt8    .CA A.T AC. .A. C.A A.A ATT ... C.A A.T ..A G.C ... T.. T.. G.C ... T.G TGC ..A ...
LS        .CA A.T AC. .A. C.A A.A ATT ... C.A A.T ..A G.C ... T.. T.. GC. ... T.G TGC ..A ...
SGG       .CA A.. CCC .A. C.A A.A ATT ... C.A A.T ..A G.C ... T.. T.. GC. ... T.G TGC ..A ...
F5        .CA A.C CCC .A. C.A A.A GTT ... C.A A.T ..A G.C ... T.. T.. G.. C.. T.A TGC ..A ...
F6        .CA A.T AC. .A. C.A A.A ATT ... C.A A.T ..A G.C ... T.. T.. GC. ... T.G TGC ..A ...
FM2       .cA A.C CCC .A. C.A A.A ATT ... C.T A.T ..A G.C ... T.. T.. G.C C.. T.A TGC ..A ...
HS        .CA A.. CCC .A. C.a A.A ATT ... C.A A.T ..A G.C ... ... T.. G.C C.. T.G TGC ..A ...
F5+6      .CA A.C CCC .A. C.A A.A ATT ... C.T A.T ..A G.C ..G T.. T.. G.. C.. T.G TGC ..A ...
FM3       .CA A.C CCT .A. ..A A.A GTT ... C.A A.T ..A G.C ... T.. T.. G.. C.. T.A TGC ..A ...
FM1       .CA A.C CCT .A. ..A A.A GTT ... C.A A.T ..A G.C ... T.. T.. G.. C.. T.A TGC ..A ...
SO        .CA A.C cC. TAC C.A A.A ATT ... C.A A.T ..A G.C ... ... T.. .CC C.G T.A TGT ..A ..A
SP        .CA A.C CA. .AC C.A A.A ATT ... C.A A.T ..A G.C ... T.. T.. .CC C.. T.A TGC ..A ...

Bovine    CTC CCT CTA ATA CTA ATA GCT AGC CAA CAT CAT CTA TCA AAA GAA AAC CTA ACC CGA AAA AAA
pSgmt8    ..T ... T.. ... G.. C.. ..A ..T ... A.. ..C ... AA. TC. ... CCT A.. CAT ... ... CGC
LS        ..T ... T.. ... G.. CCA ..T ..T ... A.. ..C ... AA. TC. ... CC. ..C CA. ... ... CGC
SGG       ..T ... T.. ... G.. CCA ..A ..T ... A.. ..C ... AA. TC. ... CCT A.. CAT ... ..G CGC
F5        ..T ..A ... ... G.T C.. ..A ..T T.. A.. ..C ... AA. CT. ... CCT A.. TA. ... ... CGT
F6        ..T ... T.. ... G.. C.. ..A ..T ... A.. ..C ... AA. TC. ... CCT A.. CAT ... ... CGC
FM2       ..T ..A ... ... G.. C.. ..A ... A.. A.. ..C ... AA. TC. ..G CC. T.. CA. ... ... CGC
HS        ..A ..G ... ... G.. C.. ..A ..T ... A.. ..C ... AA. TC. ... CC. A.C CA. ... ... CGT
F5+6      ..T ..A ... ... G.. C.. ..A ... ... A.. ..C ... AA. CT. ... CC. ... CA. ... ... CGT
FM3       ..T ..A ... ... GCT C.. ..A ... ... A.. ..C ... AA. TC. ... CC. ... CA. ... ... CGT
FM1       ..T ..A ... ... GCT C.. ..A ..T ... A.. ..C ... AA. CT. ..G CCA A.. TA. ... ... CGT
SO        ..A ..A ... ... G.. C.. ..A ... ... A.C ..C ... AA. TC. ..G CC. ..T CA. ... ... CGT
SP        ..C ..A ... ... G.. C.. ..A ... ... A.C ..C ... AA. TC. ..G CCA ..T CA. ... ... CGG

Bovine    CTA TTT ATT ACT ATG CTG ATC TCA CTA CAA CTA TTC CTA ATT ATA ACC TTT ACC GCC ATG GAA
pSgmt8    A.. ... T.A .TA .CC ..T TCT ATT ... ... .C. ... ... ..C C.T ..A ... ..A ..A .CA A.T
LS        A.. ... T.A .TA .CA ..T TCT ATC T.. ... AC. ..G T.. ..C C.T ..A ... G.A ..G .CA A.C
SGG       A.. ... T.A .TA .CC ..T TCT ATT ... ... AC. ... ... ..C C.T ..A ... ..A ..A .CA A.T
F5        G.G ... T.A .TA .CC ..T TCT ATC T.. ... AC. C.. ..C ..C C.C ..A ..C ..A ..A TCT AGC
F6        A.. ... T.A .TA .CC ..T TCT ATT ... ... AC. ... ... ..C C.T ..A ... ..A ..A .CA A.T
FM2       A.. ... T.A .TA .CT A.T TC. ATT T.. ... AC. ... ... ..C C.T ..A ... T.A ..A ..T A.C
HS        A.. ... T.A .TA .CC ..T TCT ATC T.. ... AC. ..A T.. ..C C.C ..A ... ..A ..A .CA A.C
F5+6      G.. ... T.A .TA .CT A.T TCT ATT ... ... AC. ... ... ..C C.T ..A ... T.A ..A ..C A.C
FM3       G.. ... T.A .TA .CT A.T TCT ATT ... ... AC. ... ... ..C C.T ..A ... T.A ..A ..T A.C
FM1       G.G ... T.A .TA .CC ..T TC. ATT T.. ... AC. ... ..C ..C C.C ..A ..C ..A ..A TCT A.C
SO        A.T ... C.A .TA .CT ..T TCT ATC ... ... ACC C.. ... ..C C.T ..A ..C G.A ..A TCA A.C
SP        A.. ..C C.A .TA .CC ..C TC. ATC ... ... AC. C.. ..G ..A C.. ..A ... ..A ..A GCA A.C


Bovine    CTA ATC TTA TTT TAT ATT CTA TTT GAA GCA ACA CTA GTC CCA ACA CTC ATT ATT ATT ACC CGA
pSgmt8    T.C .C. C.. ... ... ... A.. ... ... ... ..T TTT ... ... ..C ..A ... ... ... ..T ...
LS        T.C .C. C.. ... ..C ... A.. ... ..C ..T T.. A.. ... ..C ..A ... ... ... ..T ...
SGG       T.C .C. C.. ... ... ... A.. ... ... ..T ..T T.. ... ... ..C ..A ... ... ... ..T ...
F5        ... .C. T.. ... ... ..C A.. ... ..G ..C ..C ..T A.. ... ..C ..A ..C ... ... ..T ..C
F6        T.C .C. C.. ... ... ... A.. ... ... ..T ..T T.. ... ... ..C ..A ... ... ... ..T ...
FM2       T.. .C. C.. C.. ... ... A.. ... ... ..C ..C T.. A.. ... ..C ..A ... ... ... ..T ..C
HS        T.C .CT ... ... ... ... A.. ... ... ..T ..T ..G ... ... ..T ..A ... ... ... ..T ...
F5+6      ... .C. C.. C.. ... ... A.. ... ... ..C ..C ... A.. ... ..C ..A ... ... ... ..T ..C
FM3       ... .A. C.. C.. ... ..C A.. ... ..G ..C ..C ... A.. ... ..C ..A ... ... ... ..T ..C
FM1       ... .C. ... ... ... ... A.. ... ... ..C ..T ..T A.. ... ..C ..G ..C ... ... ..T ..C
SO        ... .C. C.. ... ..C ... A.. ... ..G ..T ..C ..G ... ..T ..C ..A ..C ... ..C ..T ...
SP        ..C .C. T.G ... ... ... A.. ... ... ..T ..A ... A.. ... ..C ... ... ... ... ..T ..T

Bovine    TGA GGA AAC CAA ACA GAA CGC CTA AAC GCC GGA CTC TAT TTC CTA TTC TAT ACA CTA GCT GGC
pSgmt8    ... ..G ..T ... G.. ... ..A .A. TGG ..A ... ACA ..C ... T.. ... ..C ..C ... ..A A..
LS        ... ..G ... ... G.. ... ..A ... .C. ..A ... ACA ..C ... T.. ... ... ..C T.. ..A A..
SGG       ... ..G ..T ... G.. ... ..A ... .C. ..A ... ACA ..C ... T.. ... ..C ..C ... ..A A..
F5        ..G ... ... ... CA. ... ..A ... .C. ..A ... ACA ..C ..T T.. ... ... ..C ... ..A A..
F6        ... ..G ..T ... G.. ... ..A ... .C. ..A ... ACA ..C ... T.. ... ..C ..C ... ..A A..
FM2       ... ... ... ... CA. ... ..A ... .C. ..A ..G ACA ... ..T T.. ..T ... ..T ... ..A A..
HS        ..G ... ... ... G.. ... ..A ... .C. ... ... ACA ..C ..T T.. ... ... ..C ... ..A A..
F5+6      ... ... ... ... CA. ... ..A ... .C. ..A ..G ACA ... ..T T.. ..T ... ..C ... ..A A..
FM3       ..G ... ... ... CA. ... ..A ... .C. ..A ... ACA ..C ..T T.. ... ... ..C ... ..A A..
FM1       ..G .A. ... ... CA. ... ..A ... .C. ..A ... ACA ..C ..T T.. ..T ... ..C ... ..A A..
SO        ..G .A. ... ... G.. ... ..A ... .C. ..A ... ACT ..C ..T T.. ..T ... ..C ... ..A A..
SP        ... ..C C.T ... G.. ... ..A ... .C. ..A ..C ACA ..C ..T T.. A.T ... ..C ... ..A A..
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FIGURE 3. Continued.

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Bovine  TCC CTA CCC CTA TTA GTC GCA CTA ATT TAT ATC CAA AAC ACA GTA GGA TCC CTA AAT TTC CTA
pSgmt8  ..A T.. ..A .:. ... A.T ..C A.T C.A ..C C.G A.C TC. .A. AAC CAC CAT TC. TC. A.T A..
LS      ..A T.. ... ... ... A.T ..C A.C C.A ... T.T A.T .CT .A. AAC CAC CA. TC. TC. A.T A..
SGG     ..A T.. ..A ... ... A.T ..C A.T C.A ..C C.G A.C TC. .A. AAC CAC CAT TC. TC. A.T A..
F5      ... ... ..G ... ... A.T ..T A.T T.A ..C T.T A.C .C. .A. AAC CAC CA. TC. TCA A.T AC.
F6      ..A T.. ..A ... ... A.T ..C A.T C.A ..C C.G A.C TC. .A. AAC CAC CAT TC. TC. A.T A..
FM2     ... T.. ..A ... C.. A.T ..C A.T C.A ..C T.T A.T .C. .A. AAC CAC CA. TC. TC. A.T A..
HS      ... T.. ... ..G ... A.T ..C A.T T.G ..C T.T A.C .C. .A. ACC CAC CA. TC. TCC A.T A..
F5+6    ... T.. ..A ... C.. A.T ..C A.T T.A ..C T.T A.T .C. .A. AAC CAC CA. TC. TC. A.T A..
FM3     ... T.. ..G ... ... A.T ..T A.T T.A .GC T.T A.C .C. .A. AAC CAC CA. TC. TC. A.T AC.
FM1     ... ... ..A ... C.. A.T ..T A.C T.A ..C T.T AGC .C. .A. AAT CAT CA. TC. TC. A.T A.T
SO      ... ... ... ... C.. A.T ... A.C T.A ..C T.T A.T .CT .A. AAC TCC CA. TC. TCC AC. A..
SP      ... ... ... ... C.. ..A ..C A.C T.A ... T.. A.T .CT .A. AAT CAC CA. TCT TC. A.T A.T

Bovine  ATA TTA CAG TAC TGA GTA CAA CCT GTT CAT AAC TCT TGA TCT AAT GTC TTC ATA TGA CTA GCA
pSgmt8  TTA C.. ..A CTT AC. CA. .C. .AA C.A ACA ... A.A ... ..A ..C A.T ..T ..G ..G GCC ..C
LS      T.. C.. ..A CTT AC. CA. .C. .AA C.A ACA ... A.A ... ..A ..C ... ..T ..G ..G GCC ..C
SGG     T.. C.. ..A CTT ACC CA. .C. .AA C.A ACA ... T.A ... ..C ..C ..T ..T ..G ..G GCC ..C
F5      T.. C.. ..A CTT ACC CA. .C. .AA C.A ACA ... A.A ... ..C ..C ..T ..T ... ..G GCC ..C
F6      T.. C.. ..A CTT AC. CA. .C. .AA C.A ACA ... A.A ... ..A ..C A.T ..T ..G ..G GCC ..C
FM2     T.. C.. ..A CT. ACC CA. .C. .AA T.A ACA ... A.A ... ..A G.C ... ..T ..G ..G GCC ..C
HS      T.. C.. ..A CT. ACC CA. .CC .AA C.A ACA ... A.A ... ..A ..C ..T ..T ... ... ACC ..C
F5+6    T.. C.. ..A CT. AC. CA. .C. .AA T.A ACA ..T A.A ... ..A ..C ..T ..T ..G ..G GCC ..C
FM3     T.G C.. ..A CTT ACC CA. .C. .AA C.A ACA ... ..A ... ..C ..C ..T ..T ..G ..G GCC ..C
FM1     T.. C.. ..A CTT ACC CA. .C. .AA C.A ACA ... A.A ... ... ... ... ..T ... ..G GCC ..C
SO      C.C C.. ..A .TA ACC CAG .C. .AT C.T ACA ... A.A ... ..A ..C A.. ..T ... ..G GCC ..C
SP      C.. C.. ..A ATT ATC CA. .C. .AA C.A ACA ... A.A ... ..C ..C A.T ..T ..G ... ACC ..C

Bovine  TGT ATA ATA GCT TTC ATA GTA AAA ATA CCA CTA TAT GGC CTC CAC CTT TGA CTA CCT AAA GCT
pSgmt8  ... C.. C.T ..C ..T ... ..T ... ... ..T ... ... ..A ..T ... ..A ... ..C ..A ... ...
LS      ... C.. C.T ..C ..T ... ..T ... ... ..C ... ... ..A ..T ... ..A ... ..C ..A ... ...
SGG     ..C C.. C.G ..C ..T ..G ..T ... ... ..C ..G ... ..G ..T ... ..A ..G ..C ..C ... ...
F5      ..C C.. C.. ..C ..T ... ..T ... ... ..C ..G ... ..G ..T ... ..A ... ..C ..A ... ..C
F6      ... C.. C.T ..C ..T ... ..T ... ... ..T ... ... ..A ..T ... ..A ... ..C ..A ... ...
FM2     ..C C.. C.. ..C ..T ... ..T ... ... ... ... ... ..A ..T ... ..G ... ..G ..A ... ..C
HS      ..C C.. C.. ..C ..T ... ..T ... ... ... ... ... ... ..T ..T ... ... ... ..A ... ...
F5+6    ..C C.. C.. ..C ..T ... ..T ... ..G ... ... ... ..A ..T ... ..A ... ..G ..A ... ..C
FM3     ..C C.. C.G ..C ..T ... ..T ... ..G ... ..G ... ..G ..T ... ..A ... ..G ..A ... ..C
FM1     ..C C.. T.. ..C ..T ... ..T ... ..G ... ... ... ..G ..T ..T ..A ... ..C ..A ... ...
SO      ..C C.. ..G ..C ... ... ... ... ... ... ..T ..C ..A ..T ... ..G ... ... ..C ..G ..C
SP      ..C C.. C.T ... ... ... ..T ... ... ..C T.. ... ..A ..T ... ..A ... ..C ..A ... ..C

Bovine  CAC GTA GAA GCC CCC ATC GCA GGC TCC ATA GTC CTT GCA GCA GTT CTA CTA AAA CTA GGG GGG
pSgmt8  ... ... ... ..T ..A ..T ... ..T ..A ... ... ..A ..C ..T A.. ..C ... ..G ..T ..T ..T
LS      ... ... ... ..T ..A ..T ... ..T ..A ... ..T ..G ..T ..C A.. ..T ... ..G ..T ..A ..T
SGG     ..T ... ... ... ..A ..T ... ..A ..A ... ..T T.A ..T ..C A.. ..C ... ..G ..T ..A A.C
F5      ... ... ..G ..T ..A ..T ... ..A ..A ... ..C ..A ..C ..C A.. ..C ... ... ..T ... ..C
F6      ... ... ... ..T ..A ..T ... ..T ..A ... ... ..A ..C ..T A.. ..C ... ..G ..T ..T ..T
FM2     ... ... ..G ..T ..A ..T ... ..A ..A ... ..T ..A ..T ..C A.. ..C ... ... ..T ..A A.C
HS      ..T ... ... ... ..A ..T ... ..A ..A ... ..T ..C ..C ..C A.C ..C ... ... ..T ..C ..T
F5+6    ... ... ... ... ..A ..T ... ..A ..A ... ..T T.A ..T ..C A.. ..C ... ... ..T ..A A.C
FM3     ... ... ..G ..T ..A ..T ... ..A ... ... ... ..A ..C ..C A.C ..C ... ... ..T ..A ..C
FM1     ... ..T ..G ..T ..A ..T ..T ..G ..A ... ..T ..A ..C ..C A.. ..C ... ... ..C ..A ..C
SO      ... ... ... ..T ..A ..T ... ..A ..A ... ..A ..A ..C ..C A.. ..C ... ... ..T ..A ..C
SP      ..A ... ... ..T ..A ..T ... ..G ..A ..G ..T ..A ..C ..T A.C ..T ... ... ..C ... ..T

Bovine  TAC GGT ATG CTA CGA ATC ACA CTA ATT CTA AAC CCT ATG ACC GAC TTT ATA GCA TAC CCA TTC
pSgmt8  ... ... ..T A.. ... ..T ... A.. TCA ..T .TA ..A ..A ... CCA AAA C.C TAC ... ..C ...
LS      ... ... ..T A.. ... ..T ..T ACG TCA ..T .TA ..A ..A ... CCA AAA C.C TAC ..T ..C ...
SGG     ..T ... ..T A.. ... ... ... ACG T.A ... .TA ..A ..A ... CCA AAA C.C TAT ... ..C ..T
F5      ... ..C ..C A.. ... ... ... A.. T.A ... .CA ..A T.. ..T CCA AAA C.. TAC ... ... ..T
F6      ... ... ..T A.. ... ..T ... A.. TCA ..T .TA ..A ..A ... CCA AAA C.C TAC ... ..C ...
FM2     ..T ... ..T A.. ... ..T ... A.. T.A ... .TA ..A ..A C.. CCA AAA C.. TAC ..T ... ..T
HS      ..T ... ..T A.. ... ... ... A.G T.A ... .TA ..A ..A ... CCA AAA C.C TAC ..T ... ..T
F5+6    ..T ... ..T A.. ... ... ... A.G T.A ... .TA ..A ..A ... CCA AAA C.. TAT ..T ... ..T
FM3     ... ..C ..C A.. ... ... ... A.. T.A ... .CA ..A T.A ..T CCA AAA C.. TAC ... ... ..T
FM1     ..T ..C ..T A.. ... ..T ... AC. T.A ... .TA ..A ..A ... CCA AAA C.. TAC ... ... ..T
SO      ... ..C ..C A.. ... ... ... A.. TCA T.. .C. ..A ..A ... CCA AAA T.. TAC ... ... ..T
SP      ..T ... ..T A.. ... ..T ... AC. TCA ... GC. ..A C.T ..G CCA AAA C.T TAC ... ... ..T
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FIGURE 3. Continued.

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Bovine  ATT ATA CTC TCC CTA TGA GGC ATA ATT ATA ACC AGC TCA ATC TGC CTC CGT CAA ACG GAC CTA
pSgmt8  ..A ..T T.A G.. ..C ... ..A ..C G.C ... ..A ..T ... ..T ... A.A ..A ... ..A ... ...
LS      ..A ..T T.A G.. ..C ... ..G ..C G.C ... ..A ..T ... ..T ... A.A ..A ... ..A ... ...
SGG     ..A ..C ..A G.. ..C ... ..A ..T G.C ..G ..A ..T ... ... ... A.A ..A ... ..A ... ...
F5      ..A ..T ..A G.A ..C ... ..A G.C G.C ... ..A ..T ... ... ... A.G ..A ... ..A ... T..
F6      ..A ..T T.A G.. ..C ... ..A ..C G.C ... ..A ..T ... ..T ... A.A ..A ... ..A ... ...
FM2     ..A ..C ..A G.A ..C ... ..A ..C G.C ... ..A ..T ... ... ... A.A ..A ... ..A ... ...
HS      ..A ..C ..A G.A ..C ... ..A ..C G.A ... ..A ... ... ... ... A.A ..A ... ..A ... ...
F5+6    ..A ..C ..A G.A ..C ... ..A ..T G.C ..G ..G ..T ... ... ... A.A ..A ... ..A ... ...
FM3     ..A ..C ..A G.A ..C ... ..A ..C G.C ..G ..A ..T ... ..T ... A.G ..A ... ..A ... T..
FM1     ..A ..C ..A G.A ..C ... ..A ..C G.C ... ..A ..T ... ... ... A.A ..A ... ..A ... ...
SO      ..G ..C ..A G.A ... ..G ..T ..T G.. ... ..A ... ... ... ... A.G ..A ... ..A ... ...
SP      ..A ..C ..T G.A ..T ... ... ..T G.. ... ..A ... ..G G.A ..T A.A ..A ... ..A ... ...

Bovine  AAA TCA CTC ATC GCA TAC TCC TCT GTA AGC CAC ATA GCA CTC GTT ATC GTA GCC ATC CTT ATC
pSgmt8  ... ... ... ..T ..C ..T ..A ... ..T ... ... ... .G. T.A ..A G.T .C. ..A TG. ..A ...
LS      ... ... ... ..T ..C TAT ..A ... ..T ... ... ... .G. T.A ..A G.T .C. ..A TG. ..A ...
SGG     ... ... ..T ... ..C ... ..A ... ..T ... ... ..G .G. T.A ..A G.T .C. ... TG. ..A ...
F5      ... ... ..A ... ..C ... ..A ... ..T ... ... ... .G. ..G ..A ..T .C. ... TG. ... ...
F6      ... ... ... ..T ..C ..T ..A ... ..T ... ... ... .G. T.A ..A G.T .C. ..A TG. ..A ...
FM2     ... ... ..T ..A ..C ..T ..A ... ..T ... ... ... .GG T.A ..A ..T .C. ... TG. ..A ...
HS      ... ... ... ..T ..C ... ..A ... ..T ..T ..T ..G .G. T.A ..A ..T .C. ... TG. ..G ...
F5+6    ... ... ..T ... ..C ..T ..A ... ..T ... ... ..G .G. T.A ..A ..T .C. ... TG. ..A ...
FM3     ... ... ..A ... ..C ... ..A ... ..T ... ... ... .G. ..G ..A ..T .C. ... TG. ... ...
FM1     ... ... ..T ... ..C ..T ..A ... ..T ... ... ... .GG T.A ..A ..T .C. ... TG. ..A ...
SO      ... ... ..T ..T ..C ... ... ..A ..C ... ... ..G .GG ..G ... ... .CG ..A TG. ..C ...
SP      ... ... ... ... ..C ... ..A ..A ..T ... ... ..G .G. ..A ..C ..T .C. ... TG. ..A ...

Bovine  CAG ACA CCT TGA AGC TAC ATA GGA GCA ACC GCC CTT ATG ATT GCC CAC GGC CTC ACA TCC TCC
pSgmt8  ..A ... ..A ... ... .TT .C. ..G ..T .TA AT. ..A ..A ... ... ..T ... ... ..T ..A ..A
LS      ..A ... ..A ... ... .TT .C. ..G ..T .TA AT. ..A ..A ... ... ..T ... ... ..T ..A ..A
SGG     ..A ... ..A ... ... .TT .C. ..G ..C .TA AT. ..A ..A G.C ..A ..T ... ... ..T ..G ..A
F5      ..A ... ..A ... ... .TT .C. ... ..C .TA ATT T.A ..A ..C ..A ..T ... ..A ..T ... ..A
F6      ..A ... ..A ... ... .TT .C. ..G ..T .TA AT. ..A ..A ... ... ..T ... ... ..T ..A ..A
FM2     ..A ... ..A ... ... .TT .CC ..G ..C .TA ATT T.A ..A ... ..A ..C ... ..A ..C ..A ..A
HS      ..A ... ..A ... ... .TT .C. ..G ..C .TA AT. ..A ..A ... ..A ..T ... T.A ..T ..A ..A
F5+6    ..A ... ..A ... ... .TT .CC ... ..C .TA AT. T.A ..A ..C ..A ..C ... ..A ..T ..A ..A
FM3     ..A ... ..A ... ... .TT .C. ... ..C .TA ATT T.A ..A ..C ..A ..T ... ..A ..T ... ..A
FM1     ..A ... ..A ... ... .TT .CC ..G ..C .TA ATT T.A ..A ... ..A ... ... ..A ..C ..A ..A
SO      ..A ... ..A ... ... .T. .C. ..G ..T .TA ATC ..A ..A ..C ..A ... ... ..T ... ... ...
SP      ..A ... ..A ... ... .T. .C. ..G ..C .TA ATT ..A ..A ..C ..A ..T ..A ..C ..T ..A ..A

Bovine  ATA CTT TTC TGT CTA GCA AAC TCA AAC TAC GAA CGA ATC CAC AGC CGA ACC ATA ATT CTA GCT
pSgmt8  ... T.A ..T ..C ... ... ... A.. ... ... ... ... .C. ... .C. ... ..A ... G.A CTT ..C
LS      ... ..A ..T ..C ... ... ... A.. ... ... ... ... .C. ... .C. ... ..A ... G.A CTT ..C
SGG     ... ..G ... ..C T.G ... ... A.. ... ... ... ... .C. ... .C. ... ..A ... G.G CTC ..A
F5      ... TTA ... ..C T.. ... ... A.T ... ... ... ... .C. ..T .C. ... ..A ... ..G CTC ..C
F6      ... T.A ..T ..C ... ... ... A.. ... ... ... ... .C. ... .C. ... ..A ... G.A CTT ..C
FM2     ..G ..A ... ..C T.. ... ... A.. ... ... ... ... .C. ... .C. ... ..A ... ..G CTT ..C
HS      ... ..A ... ..C ... ... ... A.. ... ... ... ..G ... .C. ... .C. ... ..A ... G.G CTT ..C
F5+6    ... ..A ... ..C T.. ... ... A.. ... ... ... ... .C. ... .C. ... ..A ... ..A CTT ..C
FM3     ... T.A ... ..C T.. ... ... A.T ... ... ... ... .C. ... .C. ... ..A ... ..G CTT ..C
FM1     ... T.A ... ..C T.. ... ... A.T ... ... ... ... .C. ... .C. ... ..A ... ..G CTT ..C
SO      ... T.A ... ..C T.. ... ... A.. ... ... ... ... .C. ... .C. ... ..G ... ... ACT ..C
SP      ... ..A ... ..C ..G ... ... A.. ... ..T ... ..T .C. ... .C. ... ..A ... ..G CTT ..C

Bovine  CGA GGC CTA CAA ACG CTC CTT CCA CTA ATA GCC ACC TGA TGA CTA CTA GCA AGT CTA ACC AAC
pSgmt8  ..C ... T.C ... .TT A.T T.. ... ... ... T.A ... ... ... ..T T.. ... .A. T.. ... ...
LS      ..C ... T.C ... .TT A.T T.. ... ... ... T.A ... ..G ... ..T T.. ... .A. T.. ... ...
SGG     ..C ... T.C ... .TT A.T T.. ... ... ... T.A ... ..G ... ..T A.. ... .A. T.. ..T ..T
F5      ... ..T T.C ... .TT A.T T.. ... ... ... T.A ... ... ... ..T A.. ... .AC ... ... ...
F6      ..C ... T.C ... .TT A.T T.. ... ... ... T.A ..A ..G ... ..T A.. ... .AC ... ... ..T
FM2     ..C ..T T.C ... .TT A.T T.C ... ... ... T.A ..A ..G ... ..T A.. ... .AC T.. ... ..T
HS      ..C ..T T.C ... .TT A.T T.. ... ... ... T.A ... ... ..G ..T A.. ... .AC T.. ... ..T
F5+6    ..C ..T T.C ..G .TT A.T T.. ... ... ... T.A ... ..G ..G ..T A.. ... .A. T.. ... ...
FM3     ..C ..T T.C ... .TT A.T T.. ... ... ... T.A ... ... ... ..T A.. ... .AC ... ... ...
FM1     ..C ..T T.C ... .TT A.T T.. ... ... ... T.A ... ... ... ..T A.. ... .AC ... ... ...
SO      ..C ..A T.T ... .TC A.T ..C ... ... ..G T.. ... ... ... ..T C.. ... .AC ... ... ..T
SP      ... ..A T.C ... .T. A.T ... ... ..T ... T.A ... ..G ... ..T C.. ... .AC T.. ..T ...
```

FIGURE 3. Continued.

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Bovine    TTA GCT CTA CCC CCA ACA ATC AAC TTA ATT GGA GAA CTA TTT GTA GTA ATG TCA ACC TTT TCA
pSgmt8    A.. ..A ..G ... ..G T.. ..T ..T C.. ..A ..G ... ..T C.C A.C A.T G.T ... TTA ..C AAC
LS        A.. ..A ..G ... ..G T.. ..T ..T C.. ..A ..G ... ..T C.C A.C A.T G.T ... TTA ..C AAC
SGG       A.G ..A ... ... ... T.. ..T ... C.. ..A ... ... T.. C.. A.C A.T G.T ... CTA ..C AAC
F5        A.. ..A ..G ... ..G T.. ..T ..T C.. ..G ..G ... ..T C.C A.C A.T G.T ... TTA ..C AAC
F6        A.. ..C T.. ... ... T.. ..T ... C.C ..A ..C ... ..T C.A A.C A.T G.. ..C TTA ... AAC
FM2       A.. ..C T.. ... ... T.. ..T ... C.C ..A ..G ... ..T C.C A.C A.T G.T ... TTA ..C AAC
HS        A.G ..A ... ... ..C T.. ... ... C.G ..G ..A ... ..A C.C A.C A.T G.T ... CTG ... AAC
F5+6      A.. ..C T.. ..A ... T.. ..T ... C.T ..A ..C ... ..T C.. A.C A.T G.A ..C TTA ... AAT
FM3       A.. ..C ..G ... ..C T.. ..T ..T C.C ..G ..C ... ..T C.. A.C A.T G.A ..C CTA ..C AAC
FM1       A.. ..C ..G ... ..C T.. ..T ..T C.C ..A ..C ... ..T C.. A.C A.T G.A ..C CTA ..C AAC
SO        A.. ..C ..T ... ... T.. ..T ... C.G ..G ..C ... ..T C.. A.T A.T G.C ..C CTA ..C AAC
SP        A.. ..A ... ..A ... T.. ..T ... C.. ..A ... ... ..T C.. A.C A.C ..T ... CTA ..C AAC

Bovine    TGA TCT AAC ATT ACA ATT ATT CTA ATA GGA GTA AAT ATA GTA ATC ACC GCC CTA TAT TCT CTA
pSgmt8    ... ... ... C.A ... ... T.A T.. .C. ... C.. GGA .CC C.. ..T ..A ..A GC. ..C ..A ..T
LS        ... ... ... C.A ..T ... T.A T.G .C. ... C.G GGA .C. C.. ..T ..A ..A GC. ..C ..A ..C
SGG       ... ... .G. CCC ..T ..C T.A T.. .C. ... C.. GGA .CG C.. ..T ..A ..A G.. ... ..A ..T
F5        ... ... ... CCA ..T ... T.A T.G .C. ... C.G GGA .C. C.. ..T ..A ..A GC. ..C ..A ..T
F6        ... ... ... CCA ..T ..C T.A T.. .C. ..T C.G GGA .C. C.. ..T ..A ..A A.. ... ..A ..C
FM2       ... ... ... C.A ... ... T.A T.. .C. ... C.. GGA .CC C.. ..T ..A ..A GC. ..C ..A ..T
HS        ... ..G .G. CCA ... ..C T.A T.. .C. ... C.G GGA .CG C.. ..T ..A ..A GC. ..C ..A ..T
F5+6      ... ... ... CCA ..T ... T.A T.. .C. ... C.G GGA .C. C.. ..T ..A ..A A.. ... ..A ..C
FM3       ... ..C ... GCA ..T ... T.A T.. .C. ... C.G GGA .C. C.. ..T ..G ..A GC. ..C ..A ..T
FM1       ... ..C ... GCA ..T ... T.A T.. .C. ... C.G GGA .C. C.. ..T ..G ..A GC. ..C ..A ..T
SO        ..G ..C ... CCA ... ..C C.A T.. .C. ..T C.. GGA .C. C.. ... ..A ..A A.. ... ..A ..T
SP        .AG ... .G. CCA ..T ..C T.A T.. .CG ... C.. GGA .C. C.. ... ..A ..A A.. ... A.A ..T

Bovine    TAC ATG CTA ATT ATA ACC CAA CGA GGA AAA TAT ACC TAC CAC ATT AAT AAT ATC TCG CCT TCC
pSgmt8    C.T ..A T.C C.C .CC ... .C. ... AAC ... CTC C.A ACA A.. ... ..C CT. TCT GAT ..A A..
LS        C.. ..A T.C A.C .CC ... ... ... AAT ... .TA C.A ACA A.. ..C ..C CT. TCA GAC ..C A..
SGG       C.. ..A T.C C.C .CC ..T ... ... AAT ... .TA C.A ACA A.. ..C ..C CT. TCT AAT ..A A..
F5        C.. ..A T.C A.C .CC ... ... ... AAC ... .TA C.A ACA A.. ... ..C CT. TCT GAC ..A A..
F6        C.. ..A T.C ..C .CC ... ... ... AAC ... CTC C.A ACA A.. ... ..C CT. TCT GAT ..A A..
FM2       C.T ..A T.C C.C .CT ... ... ... AAC ... CTC C.A ACA A.. ... ..C CT. TCT GAT ..A A..
HS        C.T ..A T.C C.A .CC ... ... ... AAC ... CTA C.A GCA A.. ... .GC CTC TCT GAC ..C A..
F5+6      C.. ..A T.C ..C .CC ... ... ... AAT ... .TA C.A ACA A.. ... ..C .T. TCT GAC ..C A..
FM3       C.. ..A T.C ..C .CC ... ... ... AAC ... .TA C.A ACA A.. ... ..C CT. TCT GAC ..A A..
FM1       C.. ..A T.C ..C .CC ... ... ... AAC ... .TA C.A ACA A.. ... ..C CT. TCT GAC ..A A..
SO        C.T ..A T.C C.A .CC ... ..G ... AAC ... CTC C.A ACA A.. ... .GC TT. TAT GAC ..A A..
SP        C.. ..A T.T C.. .CT ... ... ... AAC ... .TA C.A ACA A.. ..C ..C TT. TCT GAC ..C ...

Bovine    TTT ACA CGG GAA AAT GCA CTC ATA TCA TTA CAC ATC CTA CCC CTA CTA CTC CTA ACC CTA AAC
pSgmt8    CA. ... ..A ... C.. CT. T.A ... GT. C.T ... C.G GCC ..A ... GCT ... A.T .TT AC. ..A
LS        CA. ... ..A ..G C.C CT. ..A ... A.. T.T ... C.A GCC ..A ... A.. ..T A.T .TT AC. ..A
SGG       CA. ... ..A ... C.C CT. T.A ... ATT C.. ... C.G GCC ..A ... A.. ..T A.T .TT AC. ..A
F5        CA. ... ..A ... C.. CTC ..A ... AT. ... ... C.. GCC ..A T.. A.T ..T A.C .TT AC. ..A
F6        CA. ... ..A ... C.. CT. T.A ... GT. C.T ... C.G GCC ..A ... GCT ... A.T .TT AC. ..A
FM2       CA. ... ..A ... C.. CT. T.A ... GT. C.T ... C.G GCC ..A ... GCT ... A.T .TT AC. ..A
HS        CA. ... ..A ... C.C CT. T.A ... AT. C.T ... C.A GCC ..G ... GC. ... A.T .TT AC. ..A
F5+6      CA. ... ..A ..G C.C CT. ..A ... A.. ..T ... C.A GCC ..A ... A.. ..T A.T .TT AC. ..A
FM3       CA. ..G ..A ... C.. CTC ..A ... AT. ... ... C.A GCC ..A T.. A.T ..T A.C .TT AC. ..A
FM1       CA. ... ..A ... C.. CTC ..A ... AT. ... ... C.A GCC ..A T.. A.T ..T A.C .TT AC. ..A
SO        CAC ... ..A ..G C.C CTT ..A ... CTT C.. ... C.T GCC ..A ... A.C ... A.T .TT GCC ..A
SP        CAC ... ..A ..G C.C CTT ..A ... G.. C.C ... C.A GCC ... T.. A.C ..T A.T .T. AC. ..A

                                                                    * HIS-tRNA
Bovine    CCA AAA ATT ATT CTA GGA CCT CTA TAC TG TAAATATAGTTTAACAAAAACATTAGATTGTGAATCTAACAA
pSgmt8    ... GCC C.A ... TC. ... TTA A.C AT. .. .T.G.....................GCA...GCC....A..
LS        ... GCC C.A ... TC. ..G ..A A.T A.. .. .T.G.....................GCA...GCC....T..
SGG       ... GCC C.A ... TC. ..C TTA A.T A.. .. .T.G.....................GCA...GCC....T..
F5        ... GCC C.A ... TC. ..C TTA A.T A.. .. .T.G.....................GCA...GCC....T..
F6        ... GCC C.A ... TC. ... TTA A.C AT. .. .T.G.....................GCA...GCC....A..
FM2       ... GCC C.A ... TC. ..C TTA A.T A.. .. .T.G.....................GCA...GCC....T..
HS        CCC .CT C.A ... TC. ..C TTA A.C A.. .. .T.G.....................GCA...GCC....A..
F5+6      ... GCC C.A ... TC. ..C TTA A.T A.. .. .T.G.....................GCA...GCC....T..
FM3       ... GCC C.A ... TC. ..C TTA A.T A.. .. .T.G.....................GCA...GCC....T..
FM1       ... GCC C.A ... TC. ..C TTA A.T A.. .. .T.G.....................GCA...GCC....T..
SO        ... GCC C.A ..C TC. ... TTA A.T A.. .. .T.G.....................GCA...GCC....A..
SP        ..T GCC C.A ..C TC. ... TTA A.C A.. .. .T.G.....................GCA...GCC....A..
```

FIGURE 3. Continued.

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                                        *SER-tRNA
Bovine      TAGAAACTCATTACCTTCTTATTTACC G AAA--------AAGTATGCAAGAACTGCTAATTCTATGCTCCCATA-TCTA
pSgmt8      C....GT.TGACT........CAA... A .GGGGTGTTTTG.AC-AC.................TACTA.TG.AGT.AA.
LS          C....GT.TCAAC........CAA... A .GGGGTGTTTTG.AC-AC.................TACTA.TG.AGT.AA.
SGG         C....GT.-CAAC........CAA... A .GGGGTGTTTTG.AC-AC.................TACTA.TG.AGT.AA.
F5          C....GT.TCAAC........CAA... A .GGGGTGTTTTG.AC-AC.................TATTA.TG.AGCCAA.
F6          C....GT.TCGACT.......CAA... A .GGGGTGTTTTG.AC-AC.................TACTA.TG.AGT.AA.
FM2         C....GT.TCAAC........CAA... A .GGGGTGTTTTG.AC-AC.................TACTA.TG.AGT.AA.
HS          C....GT.TCAATT.......CAA... A .GAGGTGTTTTG.AC-AC.................TACTA.TG.AGT.AA.
F5+6        C....GT.TCAAC........CAA... A .GGGGTGTTTTG.AC-AC.................TACTA.TG.AGT.AA.
FM3         C....GT.-CAAC........CAA... A .GGGGTGTTTTG.AC-AC.................TATTA.TG.AGT.AA.
FM1         C....GT.-CAAC........CAA... A .GGGGTGTTTTG.AC-AC.................TATTA.TG.AGT.AA.
SO          C....GT.-TAAC........CAA..A A .GGGGTGTTTTG.AC-AC.................TAC.A.TG.AGT.AA.
SP          C....GT.-TAAC........CAA... A .GAGGTGTCTTG.AC-AC..-.-............TAC.A.TG.AGT.AA.


                          *LEU-tRNA
Bovine      ATAGTATGGCTTTTTC GA ACTTTTAAAGGATAGTAGTTTATCCGTTGGTCTTAGGAACCAAAAA-ATTGGTGCAACTCC
pSgmt8      ..CC.CA.-ACC.C.T -- ..........-...AA...-A....AC...........G........-C.............
LS          ..CC.CA..ACC.C.T -- ..........-...AA...-A....AC...........G.....GA-C.............
SGG         ..CC.CA.-ACC.C.T -- ..............AA.C.-A....AC...........G.....GACC.........A...
F5          ..CC.CA..ACC.C.T -- .............GAA...-A....AC...........G........-C.............
F6          ..CC.CA..ACC.C.T -- ..........-...AA...-A....AC...........G.......CC.............
FM2         ..CC.CA.-ACC.C.T -- ..............AA...-A....AC.....................CC.............
HS          ..CC.CA.-ACC.C.T -- ..............AA...-A....AC...........CG.......CC.........A...
F5+6        ..CC.CA..ACC.C.T -- ..........-...AA...-A....AC...........G.......CC.............
FM3         ..CC.CA.-ACC.C.T -- ..........-...AA...-A....AC...........G.......CC.............
FM1         ..CC.CA.-ACC.C.T -- ..............AA...-A....AC...........G.......CC.............
SO          ..CC.CA.-ACC.C.T -- ..............AA...-A.....C...........G.......CC.............
SP          ..CC.CA.-ACC.C.T -- ..............G...-A....AA...........CG.......CC.............

                     12108
Bovine      AAATAAAAGTA
pSgmt8      ..G........
LS          ..GG.......
SGG         ..G........
F5          ..G........
F6          ..G........
FM2         ..G........
HS          ..G........
F5+6        ..G........
FM3         ..G........
FM1         ..G........
SO          ..G........
SP          ..G........
```

FIGURE 3. Continued.

tween trees obtained with alternative outgroups for the same gene and for trees obtained with the same outgroup for different genes. All of these searches recovered trees that were largely congruent with each other and with those recovered by the exhaustive search for the entire fragment (Fig. 5). Two clades can be consistently recognized: a low-2*n* group containing the LS, SGG (both $2n = 32$), F6 ($2n = 34$), and pSgmt8 sequences; and a high-2*n* group containing all three FM races ($2n = 38$–46) and the F5 ($2n = 34$) and F5+6 ($2n = 36$) races. The major differences among these topologies are (1) four alternative positions for the HS race: sister group of the remainder of the low-2*n* clade, nested within the low-2*n* clade, sister group of the high-2*n* clade, or sister group of the entire radiation; (2) alternative arrangements of LS and SGG as first and second outgroups of the F6 + pSgmt8 clade, which is recovered in every analysis; and (3) several alternative arrangements of F5, FM1, and FM3 within the high-2*n* clade, relative to each other and the consistently recovered F5+6 + FM2 clade.

The five tRNAs were combined together for similar analyses and appeared to perform poorly relative to either the entire sequence or individual protein genes. Multiple equally parsimonious trees were recovered for each outgroup, and consensus topologies failed to recover most or all of the clades regularly recovered with the other data sets. These analyses of tRNA sequences were based on equal character weighting and did not consider possible effects of secondary structure on substitution rates (Wheeler and Honeycutt, 1988;

TABLE 3. Transition/transversion (TA/TV) counts for the mtDNA fragment sequenced in this study among the 10 *Sceloporus* ingroup taxa (including pSgmt8 and *S. grammicus grammicus*) for each codon position (numbers 1, 2, 3) for each of the protein-coding genes and the unique variation for both outgroups (considered together). Values in parentheses are the percentages of each type of replacement for each codon position of each gene.

Protein-coding genes	Ingroup		Outgroup	
	TA	TV	TA	TV
COIII [66 bp]				
1	2 (3.03)			
2				
3	9 (13.64)	4 (6.06)	3 (4.55)	1 (1.52)
ND3 [350 bp]				
1	33 (9.33)	15 (4.29)	14 (4.00)	9 (2.57)
2	13 (3.71)	4 (1.14)	7 (2.00)	2 (0.57)
3	65 (18.57)	34 (9.71)	37 (10.97)	27 (7.71)
ND4L [290 bp]				
1	17 (5.86)	4 (1.38)	12 (4.14)	7 (2.41)
2	9 (3.10)	1 (0.34)	6 (2.07)	
3	71 (24.48)	20 (6.90)	31 (10.69)	20 (6.90)
ND4 [1,381 bp]				
1	114 (8.25)	28 (2.02)	59 (4.27)	19 (1.38)
2	46 (3.33)	8 (0.58)	17 (1.23)	3 (0.22)
3	318 (23.03)	138 (10.00)	147 (10.64)	105 (7.60)

Dixon and Hillis, 1993). A consideration of tRNA secondary structure will be presented in another paper, and because the skewness statistics suggest that variation in the *S. grammicus* tRNA sequences is randomly distributed with respect to genealogy when nucleotide changes are equally weighted, we excluded them from further consideration in this study.

Several frequently used character weighting options were employed in a second round of analyses on all protein sequences combined to determine if trees would converge toward a single topology (Cracraft and Helm-Bychowski, 1991). First, different weights were assigned to transitions and transversions (by a ratio of 1:2) to compensate for transition bias (Table 3). A second approach used transversions only, and the third eliminated the third base position from the analysis. In all but one case, single most-parsimonious trees were found, and all analyses recovered both the low-2*n* and high-2*n* clades containing the same taxa as those found in the first round of analyses. As with earlier analyses, the topological position of the HS race was unstable, as were relative positions of LS and SGG within the low-2*n* clade and the F5 + FM3 + FM1 clade within the high-2*n* clade.

To test for the stability of nodes in several analyses, bootstrap resampling was carried out for the entire fragment (equal character weighting) and for the three complete protein genes translated into their amino acid sequences (Fig. 5). Unless rates of change are highly unequal and/or randomized with respect to history and/or systematic bias is present in a data set, bootstrap values of >70% are probably underestimates of phylogenetic accuracy (Hillis and Bull, 1993). All clades recovered in bootstrap resampling of the entire fragment were, with one exception (at 81%), supported by values >98%, regardless of the outgroup (Fig. 5a). However, in both trees one unresolved polytomy appeared in the low-2*n* and high-2*n* clades, and the HS race was recovered as the first outgroup for both of these polytomies. Completely resolved topologies were obtained for both outgroups when the translated amino acid data matrix was used (Fig. 4), and the tree topologies were identical for both outgroups (Fig. 5b). Bootstrap proportions were <70% at two nodes on each tree (compare topologies for SP and SO in Fig. 5b),

TABLE 4. Pairwise sequence divergence values (Kimura, 1980) for the *Sceloporus* used in this study for the mitochondrial ND3 gene (above diagonal) and ND4L gene (below diagonal).

	pSgmt8	LS	SGG	F5	F6	FM2	HS	F5+6	FM3	FM1	SO	SP
pSgmt8	—	0.07	0.05	0.14	0.01	0.17	0.16	0.17	0.20	0.21	0.20	0.20
LS	0.07	—	0.10	0.12	0.07	0.16	0.16	0.15	0.18	0.18	0.21	0.19
SGG	0.10	0.08	—	0.15	0.04	0.18	0.17	0.19	0.22	0.23	0.21	0.23
F5	0.14	0.15	0.14	—	0.13	0.12	0.16	0.12	0.10	0.10	0.19	0.22
F6	0.00	0.05	0.09	0.13	—	0.15	0.15	0.16	0.19	0.20	0.19	0.19
FM2	0.14	0.14	0.16	0.08	0.13	—	0.18	0.07	0.14	0.14	0.23	0.24
HS	0.13	0.07	0.13	0.16	0.12	0.15	—	0.17	0.21	0.21	0.22	0.22
F5+6	0.14	0.15	0.16	0.10	0.13	0.05	0.16	—	0.14	0.14	0.22	0.23
FM3	0.15	0.16	0.18	0.06	0.12	0.10	0.18	0.12	—	0.01	0.22	0.25
FM1	0.14	0.15	0.17	0.07	0.13	0.08	0.15	0.09	0.05	—	0.22	0.26
SO	0.23	0.21	0.22	0.19	0.22	0.20	0.20	0.20	0.22	0.22	—	0.18
SP	0.22	0.18	0.22	0.19	0.20	0.20	0.18	0.20	0.19	0.19	0.18	—

but overall there was strong concordance for resolution of the low-2*n* clade with the topology (HS(LS(SGG(F6, pSgmt8)))) and a topology of (FM1(FM3, F5)) for the polytomy within the high-2*n* clade.

Second-order Phylogenetic Analyses

The lack of bootstrap support for many nodes in the trees resulting from the analysis of sequence data (Fig. 5a) versus those from the analysis of amino acid sequences (Fig. 5b) suggests that none of the preliminary weighting options for DNA fully recovered the phylogenetic signal in the mtDNA protein sequences. We therefore evaluated these sequences (combined) for additional resolving power by considering all 12 base substitutions and weighting these differentially based on their observed frequencies in the study taxa. Substitution frequencies were inferred by two methods, using various options in the Chart menu of MacClade. First, the average frequencies of change between states were calculated for a single tree input from a preliminary PAUP search (the SP topology in Fig. 5). The second approach generated 100 randomly joined trees over which minimum, average, and maximum frequencies were estimated. These estimates served as the basis for the derivation of two asymmetrically weighted matrices for additional PAUP analyses (Table 6).

Specific character weights for the PAUP matrices were derived as follows. First, the reciprocal of each frequency was calculated for all relevant pairwise frequencies in each matrix (single tree and averages for 100 randomly joined trees) and converted to a whole number by multiplying the quotient by 1,000. For example, the character

TABLE 5. Pairwise sequence divergence values for the mitochondrial ND4 gene (Kimura, 1980) for the *Sceloporus* used in this study (above diagonal) and sequence differences provided by PAUP (below diagonal).

	pSgmt8	LS	SGG	F5	F6	FM2	HS	F5+6	FM3	FM1	SO	SP
pSgmt8	—	0.07	0.10	0.14	0.00	0.14	0.13	0.14	0.15	0.14	0.23	0.22
LS	15	—	0.08	0.15	0.05	0.14	0.07	0.15	0.16	0.15	0.21	0.18
SGG	19	24	—	0.14	0.09	0.16	0.13	0.16	0.18	0.17	0.22	0.22
F5	34	38	34	—	0.13	0.08	0.16	0.10	0.06	0.07	0.19	0.19
F6	0	15	19	34	—	0.13	0.12	0.13	0.12	0.13	0.22	0.20
FM2	34	37	35	21	34	—	0.15	0.05	0.10	0.08	0.20	0.20
HS	32	19	35	40	32	39	—	0.16	0.18	0.15	0.20	0.18
F5+6	35	38	36	27	35	15	40	—	0.12	0.09	0.20	0.20
FM3	35	40	42	16	35	27	45	31	—	0.05	0.22	0.19
FM1	34	38	39	19	34	22	39	24	14	—	0.22	0.19
SO	53	52	49	48	53	49	50	51	54	53	—	0.18
SP	51	45	51	47	51	51	46	50	48	49	45	—

```
ND3          8970
Bovine       MNLMLA-LLTNFTLATLLVIIAFWLPQLNVYSEKTSPYECGFDPMGSARLPFSMKFFLVAITFLLFDLEIALL
Xenopus      .TATI--.MIAM..S.I.A.LS.....MTPDM..L.........L..M......R...I.IL..........
Chicken      ..TLTFM.SLS.L.SAA.TTMN...A.MAPDT..L.........L........IR......L..........
pSgmt8       ...TTM-.IASLMVSS..IM.S......YPDT..L.........L.N......LR......L..........
LS           ...TTM-.IASLMVSS..IM.S......YPDT..L.........L.N......LR......L..........
SGG          ...TTM-.IASLMVSS..IM.S.....LYPDT..L.........L.N......LR......L..........
F5           ...TTM-.IFSLMVSL..IM.G.....PCPDT..L.........L.N......LR......L..........
F6           ...TTM-.IASLMVSS..IM.S......YPDT..L.........L.N......LR..................
FM2          ...TTM-.IFSLMISL..ILVS......YPDT..L.........L.N......LR......L..........
HS           ...TTM-.IFSLMVSL..IL.S......YPDL..L.........L........LR......L..........
F5+6         ...TTM-.IFSLMISL..ILLS......YPDT..L.........L.N......LR......L..........
FM3          ...STM-.IFSLMISL..ILV......PYPDT..L.........L.N......LR......L..........
FM1          ...STM-.IFSLMISL..ILV......PYPDT..L.........L.N......LR......L..........
SO           ...TTM-..ISLIISA..IL.S......YPDT..L.........L.N......LR......L..........
SP           ...TTM-..ISLMISA..IL.S.....FHPDT..L.........L........LR......L..........

                                                                9753   ND4L  10239
Bovine       LPLPWASQTANLNTMLTMALFLIILLAVSLAYEWTQKGLEWTEY       MSMVYMNIMMAFTVSLVGLLMYR
Xenopus      ..F...A.LNTPSIVILW.ALILT..TLG.I...L.G....A.W       .TLIHFSFCS..ILG.T..ALN.
Chicken      ......I.L.HPMMT.TW.TSI.A..TFG.I.....G....A.*       ..PLHFSFYS...F.SL..AFH.
pSgmt8       .......KLKKSTLTM.LVTIILL..TLG.I.....G....A.L       .LPMHFTLNST.IL.IM.MSIH.
LS           .......KLK.PTLTM.LVTTILL..TIG.I...A.G....A.L       .LPMHFTLNST.IL.IM.MSIH.
SGG          .......NLK.PTLTM.LVTIILL..TIG.I...A.G....A.L       .LPMHFMLNST.IL.IM.MSLH.
F5           .......NLK.PTLTMMFVTTILL..TIG.I...S.G....A.L       .LPMHFTLNST.IL.IM..S.H.
F6           .......NLK.PTLTM.LVTIILL..TLG.I.....G....A.L       .LPMHFTLNST.IL.IM.MSIH.
FM2          .......NLK.PTLTMAFVTTILI..IMG.I.....G....A.L       .LPMHFTLNST.IL.IM..S.H.
HS           .......NLE.PTLST.LVTTILL..TIG.I.....G..A.A.L       .LPMHFTLNST.IL.IM..S.H.
F5+6         .......NLK.PTLT.VFVTTILM..IIG.I.....G....A.L       .LPVHFMLNST.IL.IM..S.H.
FM3          .......NLK.PTLTMMFVTTILL..TIG.I...S.G....A.L       .LPMHFTLNST.IL.IM..S.H.
FM1          .......NLK.PTLTILFVTTILL..TIG.I...S.G....A.L       .LPMHFTLNST.IL.IM..S.H.
SO           .......NLK.PMVTMMLMTIILL..TLG.I...S.G....A.L       .LPMHFTLNST.IL.IL..S.H.
SP           .......NLK.PTVTMILTTAILL..TLG.I.....G....A.L       .LPMHFTMNST.IL.IM..S.H.

                                                                                10535
Bovine       SHLMSSLLCLEGMMLSLFVMAALTILNSHFTLASMMPIILLVFAACEAALGLSLLVMVSNTYGTDYVQNLNLL
Xenopus      .PIL.I......IL.ISIDGIV..P.HLTIY.S.IILY.ILP...P...T....NSDHYT.H...KLFS....
Chicken      T..I.A.....S....M.IPLSIWPVENQTPSFALV..LM.A.S....GT..AI..ASAR.H.S.HLH.....
pSgmt8       M....A...I.....A..IIITTFFSTNNLQTMAPT..M..A.S....ST....M.AT.R...N.NLK.....
LS           M....A...I.....A..IIITTFFSTNNLQTMAPA..M..A.S....ST....M.AT.R.H.N.NLK.....
SGG          M....A...I.....A..IIITTFFSTNSLQTMAPT.....A.S....ST....M.AT.R.H.N.DLK.....
F5           M....A...I.....A..IIITMFFSTNNLQTMAPA..M..A.S....SI....M.AT.R.H.N.NLK.....
F6           M....A...I.....A..IIITTFFSTNNLQTMAPT..M..A.S....ST....M.AT.R...N.NLK.....
FM2          M....A...I.....A..IIISTFFSTNNLQTMAPA..M..A.S....SI....M.AT.R.H.N.SLK.....
HS           M....A...I.....A..IIITTFFSTNNLQTMAPA..M..A.S....ST....M.AT.R.H.N.NLK..S..
F5+6         M....A...I.....A..IIISTFFSTNNLQTMAPA..M..ALS....SI....M.AT.R.H.N.NLK.....
FM3          M....A...I.....V..IIITTFFSTNNLQTMAPA..M..A.S....SI....M.AT.R.T.N.NLK.....
FM1          M....A...I.....V..IIITMFFSTNNLQTMAPA..M..A.S....SI....M.AT.R.H.N.NLK.....
SO           T....A...I.....A..IIT.TFFSTNNIQTMAPT..M..A.S....SV....M.AT.R.H.N.HLK.....
SP           T....A...I.....A..IITTTFFSTNNLQTMASA..M..A.S....SI......AT.R.H.S.HLK.....

ND4          10529
Bovine       MLKYIIPTIMLMPLTWLSKNNM-IWVNSTAHSLLISFTSLLLMNQFGDNSLNFSLLFFSDSLSTPLLILTMWL
Xenopus      ...ILL..L..I.S...TNKKW.L.PSL.SQ..I..LL..MWFFNQSETTHFSNY.MTI.QI........C..
Chicken      ...I.L.....L.TAL..PAKS.M.T.T.MY....ASI..HWLTPSYYPTKTLT.WTGM.QI.....V.SC.F
pSgmt8       ...V.L..L..A.TAMTT.PLYTFSLFTTYSTA.ALISLTW.KSPMNME.TFSTTQLMI.PI.A...V.SC..
LS           ...S.L..L..A.TAMTT.PMYTFNLFTMYSTI.ALISLTW.KSPMNME.TFSTTQLMI.PI.A...A.SC..
SGG          ...V.L..L..A.TAMTT.PLYTFSLFTTYSTI.ALISLTW.KLPMNTEPTFSTPQLMI.PI.A...A.SC..
F5           ...V.L..LI.A.TAMTT.PLYTFNLFTTYSTI.ALISLTW.KSSMNTEPTFSTPQLMV.PI.A...A.SC..
F6           ...V.L..L..A.TAMTT.PLYTFSLFTTYSTA.ALISLTW.KSPMNME.TFSTTQLMI.PI.A...A.SC..
FM2          ...V.L..L..A.TAMTT.PLYTFNLFTTYSMI.ALISLTW.KSSMNTEPTFSTPQLMI.PI.A...V.SC..
HS           ...V.L..L..A.TAMTT.RQYTLNLFTMYSTI.ALISLTW.KLPMNTEPTFSTPQLMI.PI.A...V.SC..
F5+6         ...V.L..L..A.TAMTT.PLYTFNLFTTYSMI.ALISLTW.KSSMNTEPTFSTPQLMI.PI.A...V.SC..
FM3          ...V.L..LI.A.TAMTT.PPYTFNLFTTYSTI.ALISLTW.KSSMNTEPTFSTPQLMV.PI.A...V.SC..
FM1          ...V.L..L..A.TAMTT.PLYTFNLFTMYSMI.ALISLTW.KSSMNTEPTFSTPQLMV.PI.A...V.SC..
SO           ...I.L..L..A.TAMVT.PTYTFSLFTMYSTT.ALISLTW.KTSMNTEPTFSTPYLMI.PI.A...T.SC..
SP           ...M.L..L..A.TAMMA.PPYTFSMFTAYSTI.ALISLTW.KTSMNTEPTFSTQHLMI.PI.A...T.SC..
```

FIGURE 4. The three entire mitochondrial protein-coding genes, ND3, ND4L, and ND4, translated into their corresponding amino acid sequences aligned against the bovine reference sequence (Anderson et al., 1982) and compared with the chicken (Desjardins and Morais, 1990) and *Xenopus* (Roe et al., 1985) sequences. Amino acid abbreviations follow the standard code; * = stop codon.

```
Bovine    LPLMLMASQHHLSKENLTRKKLFITMLISLQLFLIMTFTAMELILFYILFEATLVPTLIIITRWGNQTERLNA
Xenopus   ....II...N...N.PIS.QRT.....VF...S...A.S.T.......M..I..I............A.....
Chicken   ...I.I...G...H.PIK..RM..ST..II.P.I.LA.S.T.......S.....I...IL.......P...S.
pSgmt8    .....L...N..KS.PMH..RM.LMT.SI..P...L....TNFT....M.....................A..QW.
LS        ....VP...N..KS.PLH..RM.LMT.SI..TL..L..A.TNFT....M.....I............A...T.
SGG       ....VP...N..KS.PMH..RM.LMT.SI..T...L....TNFT....M.....................T.
F5        ....VL...N..KS.PMY..RV.LMT.SI..TL..L....SSLT....M.....I............Q...T.
F6        ....VL...N..KS.PMH..RM.LMT.SI..T...L....TNFT....M.....................A...T.
FM2       ....VL.A.IT.NQSPYTE.RM.LMTISI..T...L..S.IN.T.L..M.....I............Q...T.
HS        ....VL...N..KS.PIH..RM.LMT.SI..TL..L....TNFT....M.....................A...T.
F5+6      ....VL...N..KL.PLH..RV.LMTISI..T...L..S.IN.T.L..M.....I............Q...T.
FM3       ....AL...N..KS.PLH..RV.LMTISI..T...L..S.IN.N.L..M.....I............Q...T.
FM1       ....AL...N..KL.PMY..RV.LMT.SI..T...L....SN.T....M.....I.........E..Q...T.
SO        ....VL...N..KS.PLH..RI.LMT.SI..TL..L..A.SN.T....M.....................E..A...T.
SP        ....VL...N..KS.PLH..RM.LMT.SI..TL.ML....AN.T....M.....................

Bovine    GLYFLFYTLAGSLPLLVALIYIQNTVGSLNFLMLQYWVQPVHNSWSNVFMWLACMMAFMVKMPLYGLHLWLPK
Xenopus   .T.....................LSLYSST.T.SLNL..LLPNHIPIT.A.YSW....LL..........T......
Chicken   .I.L.....IS......SIL.LHTNT.T.HLPIIKLTHPNLPA..TSLLSS..LLM..M..A...........
pSgmt8    ..........S.....I.IL.LNSKNHHSSIML..LTQPQLT.T...I...A..LL.................
LS        .T........S.....I.IL.FNTKNHHSSIML..LTQPQLT.T.......A..LL.................
SGG       .T........S.....I.IL.LNSKNHHSSIML..LTQPQLT.........A..LL.................
F5        .T........S.....VIIL.FNTKNHHSSITL..LTQPQLT.T.......A..LL.................
F6        .T........S.....I.IL.LNSKNHHSSIML..LTQPQLT.T...I...A..LL.................
FM2       .T........S.....I.IL.FNTKNHHSSIML..LTQPQLT.T..D....A..LL.................
HS        .T........S.....I.IL.FNTKTHHSSIML..LTQPQLT.T.......T..LL.................
F5+6      .T........S.....I.IL.FNTKNHHSSIML..LTQPQLT.T.......A..LL.................
FM3       .T........S.....I.ILCFNTKNHHSSITL..LTQPQLT.........A..LL.................
FM1       .T........S.....I.IL.FSTKNHHSSIIL..LTQPQLT.T.......A..LL.................
SO        .T........S.....I.IL.FNTKNSHSSTML..LTQPHLT.T...I...A..L..................
SP        ....FI....S.......IL.FNTKNHHSSIIL..IIQPQLT.T.......T..LL.................

Bovine    AHVEAPIAGSMVLAAVLLKLGGYGMLRITLILNPMTDFMAYPFIMLSLWGMIMTSSICLRQTDLKSLIAYSSV
Xenopus   ..............I........II..SIT.S.SMKEL....LI.....I..............M......
Chicken   ..........ML...L........IM.V..LME.VSN.LH...LT.A...ALM...................
pSgmt8    ..............I........IM...MS.M...PKLY...MI.A...IV......M..............
LS        ..............I........IM...TS.M...PKLY...MI.A...IV......M..............
SGG       ..............I........IM...TL.M...PKLY...MI.A...IV......M..............
F5        ..............I........IM...ML.T.L.PKLY...MI.A...VV......M..............
F6        ..............I........IM...MS.M...PKLY...MI.A...IV......M..............
FM2       ..............I........IM...ML.M..PPKLY...MI.A...IV......M........M.....
HS        ..............I........IM.VSAL.M..TPKLY...MI.A...IV......M..............
F5+6      ..............I.....S..IM...ML.M..TPKLY...MI.A...IV......M..............
FM3       ..............I........IM...ML.T.LTPKLY...MI.A...IV......M..............
FM1       ..............I........IM...TL.M..TPKLY...MI.A...IV......M..............
SO        ..............I........IM...MS.T..TPKLY...MI.A...IV......M..............
SP        .Q............I........IM...T..A.L.PKLY...MI.A...IV....V.M..............

Bovine    SHMALVIVAILIQTPWSYM-GATALMIAHGLTSSMLFCLANSN-YERIHSRTMILARGLQTLLPLMATWWLLA
Xenopus   ...G...S.GNN...MKALT..MI.NTSD...H.A.C...KYQS...T...ALL.S...E.I....G....IS
Chicken   ...G...A.SM...Q..FS...MI...S......L......T.....T...IL..T....P.....SV.....
pSgmt8    ...G..VA.C.......FT...MI.................T.....T.T...V....F.IIF...S......
LS        ...G..VA.C.......FT...MI.................T.....T.T...V....F.IIF...S......
SGG       ...G..VA.C.......FT...MI..V..............T.....T.T...V....F.IIF...S....M.
F5        ...G..IA.C.......FT...MI.................T.....T.T...I....F.IIF...S....M.
F6        ...G..VA.C.......FT...MI.................T.....T.T...V....F.IIF...S....M.
FM2       ...G...A.C.......FT...MI.................T.....T.T...M....F.IIF...S....M.
HS        ...G...A.C.......FT...MI.................T.....T.T...V....F.IIF...S....M.
F5+6      ...G...A.C.......FT...MI.................T.....T.T...M....F.IIF...S....M.
FM3       ...G...A.C.......FT...MI.................T.....T.T...M....F.IIF...S....M.
FM1       ...G...A.C.......FT...MI.................T.....T.T...M....F.IIF...S....M.
SO        ...G...A.C.......FT...MI.................T.....T.T....T...F.II....S......
SP        ...G...A.C.......FT...MI.................T.....T.T...M....F.MI....S......
```

FIGURE 4. Continued.

weight for the A → C transversion in the single tree matrix (above the single line in the second column of Table 6) is the reciprocal of the frequency (1/125.25 = 0.008) × 1,000 = 8. The whole numbers in parentheses in Table 6 represent the actual character weights used in the PAUP matrices.

For each matrix, most-parsimonious trees were obtained by branch-and-bound

```
Bovine      SLTNLALPPTINLIGELFVVMSTFSWSNITIILMGVNMVITALYSLYMLIMTQRGKYTYHINNISPSFTRE
Xenopus     N.A.M....SP.WM..ITIMTAL.N..SW....TDLGTLL..S.....FL.....MTPE.L.A.N.TH...
Chicken     N...M.....T..MA..TIIVAL.N..SP....T.TATLL..S.T....LS....TLPS..TTTPN.N...
pSgmt8      N...M....S...M...LIIV.L.N...L..L.T.LGTL...A...H.FLT.P.N.LPTN..LSD.TH...
LS          N...M....S...M...LIIV.L.N...P..L.T.LGTL...A...H.F.T...N.LPTN..ISD.TH...
SGG         N...M....S...M...LIIV.L.N..SP..L.T.LGTL...V...H.FLT...N.LPTN..LSN.TH...
F5          N...M....S...M...LIIV.L.N..SP..L.T.LGTL...V...H.FLT...N.LPTN..LSD.TH...
F6          N...M....S...M...LIIV.L.N...P..L.T.LGTL...M...H.F.T...N.LPTN..LSD.TH...
FM2         N...M....S...M...LIIV.L.N...L..L.T.LGTL...A...H.FLT...N.LPTN..LSD.TH...
HS          N...M....S...M...LIIV.L.N..SP..L.T.LGTL...A...H.FLT...N.LPAN.SLSD.TH...
F5+6        N...M....S...M...LIIV.L.N...P..L.T.LGTL...M...H.F.T...N.LPTN..ISD.TH...
FM3         N...M....S...M...LIIV.L.N...A..L.T.LGTL...A...H.F.T...N.LPTN..LSD.TH...
FM1         N...M....S...M...LIIV.L.N...A..L.T.LGTL...A...H.F.T...N.LPTN..LSD.TH...
SO          N...M....S...M...LIIV.L.N...P..L.T.LGTL...M...H.FLT...N.LPTN.SFYD.TH...
SP          N...M....S...M...LIII.L.N..SP..L.T.LGTL...M.T.H.FLT...N.LPTN..FSD..H...

                                      11906
Bovine      NALMSLHILPLLLLTLNPKIILGPLY
Xenopus     HT..TM.LI.IIP.MMK.EL.W.LFF
Chicken     HL..T...I.M.T.I.K.EL.S.TPL
pSgmt8      HL..V..LA..A.IITK.AL.S.LII
LS          HL..TF.LA..M.IITK.AL.S..IN
SGG         HL..I..LA..M.IITK.AL.S.LIN
F5          HL..M..LA..I.IITK.AL.S.LIN
F6          HL..V..LA..A.IITK.AL.S.LII
FM2         HL..V..LA..A.IITK.AL.S.LIN
HS          HL..M..LA..A.IITK.TL.S.LIN
F5+6        HL..TF.LA..M.IITK.AL.S.LIN
FM3         HL..M..LA..I.IITK.AL.S.LIN
FM1         HL..M..LA..I.IITK.AL.S.LIN
SO          HL..L..LA..I.IIAK.AL.S.LIN
SP          HL..A..LA..I.IITK.AL.S.LIN
```

FIGURE 4. Continued.

searches and rooted with a composite outgroup (asymmetric step matrices force a rooted tree; Swofford, 1992). Both searches yielded single trees with identical topologies but different lengths (8,114 and 6,328 steps for the single-tree and random-joining tree matrices, respectively). When tested over a distribution of 1,000 randomly generated alternative trees, the shortest trees appeared to contain significant phylogenetic signal ($g_1 = -0.795$, $P < 0.01$). Support for the nodes in each tree was estimated by bootstrapping with 100 replications, utilizing *S. poinsetti* as the outgroup (Fig. 6).

Tree topologies based on these analyses are similar to those derived from translated amino acid sequences (Fig. 5b) in that they recovered both low-2*n* and high-2*n* clades, the high-2*n* clades again at especially high levels of support (bootstrap values of 82% and 98%, Figs. 6a and 6b, respectively). A single topological difference is apparent within each of these clades, however. Within the low-2*n* group, the amino acid data set recovered SGG as the sister group of the F6 + pSgmt8 clade and places LS as the first outgroup to (SGG(F6, pSgmt8)) (Fig. 5b), whereas the asymmetrically weighted mutation step matrices reversed the positions of LS and SGG; SGG is the first outgroup to (LS(F6, pSgmt8)) (Fig. 6). The HS race was recovered as the basal lineage in both sets of trees, although this position is not as strongly supported (bootstrap values of 69% and 64%, Figs. 6a and 6b, respectively) as is monophyly of other members of the low-2*n* clade. Both sets of analyses strongly support recognition of the high-2*n* clade as a monophyletic group, but these analyses differ with respect to the arrangement of the F5, FM3, and FM1 races (cf. Figs. 5b, 6). The F5+6 and FM2 races are strongly supported as a monophyletic group within the high-2*n* clade in all analyses.

DISCUSSION

Alternative Coding Methods and the Best Tree

All single trees obtained from exhaustive or branch-and-bound searches in the first round of analyses recovered the low-2*n* and high-2*n* clades, as did trees obtained from the bootstrap replications when root-

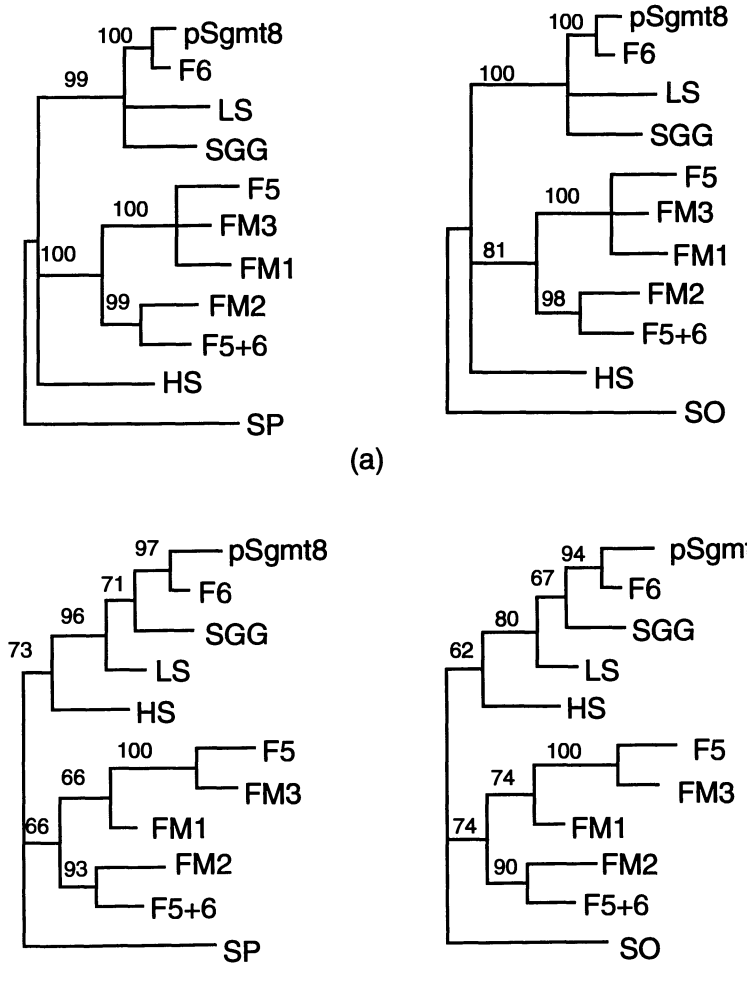

FIGURE 5. Bootstrap (with 1,000 replications) 50% majority rule consensus trees for *Sceloporus grammicus* ingroup taxa, alternatively rooted to *S. poinsetti* (SP) (left) and *S. olivaceus* (SO) (right). (a) Trees obtained for the entire fragment of sequence. (b) Trees obtained for the translated amino acid sequence of the three complete protein genes.

ed with either outgroup, albeit neither was fully resolved (Fig. 5a). Trees constructed from amino acid sequences (Fig. 5b) presented fully resolved topologies for these clades that were identical for both outgroups, and in each of these all but two bootstrap values were >70%.

Similar bootstrap values are obtained, however, for the completely resolved parsimony trees derived from the asymmetrically weighted step matrices presented in Table 6 (Fig. 6). We selected this topology as our working hypothesis to test the chromosome evolution questions posed for the *S. grammicus* complex because it appears to be the most accurate description of rates of substitution in our sequences. Li et al. (1985a, 1985b) reviewed a number of models of nucleotide substitution for protein gene sequences and showed that under many conditions both one-parameter models (those making no distinction among substitution rates for any nucleotide in any codon position) and two-parameter models (which distinguish either between transition and transversion probabilities or be-

TABLE 6. Summary of frequencies of base changes in *Sceloporus grammicus* mitochondrial protein gene sequences estimated for single input tree (above lines) and for 100 randomly joined trees (below lines; minimum, average, and maximum values from top to bottom). Numbers in parentheses are the character-weight values for the two sets of asymmetrical matrices used in PAUP analyses.

	To			
From	A	C	G	T
A	—	125.25 (8)	238.94 (4)	107.42 (9)
	—	155.66	242.32	111.22
	—	181.16 (6)	282.84 (4)	137.45 (7)
	—	202.48	308.47	153.28
C	103.73 (10)	—	19.35 (53)	374.39 (3)
	107.21	—	18.68	403.59
	125.53 (8)	—	23.21 (43)	470.63 (2)
	144.95	—	28.87	538.34
G	65.20 (15)	6.40 (167)	—	3.65 (250)
	65.57	6.32	—	2.49
	89.75 (11)	10.20 (100)	—	7.03 (143)
	115.61	14.48	—	12.12
T	52.83 (19)	310.34 (3)	11.54 (87)	—
	56.72	363.99	13.81	—
	69.04 (14)	429.67 (2)	17.85 (56)	—
	83.54	457.56	21.57	—

tween synonymous and nonsynonymous substitutions) give biased estimates of substitution rates under some conditions. Our option of equally weighting all positions would require justification of a one-parameter model of base substitution, and the amino-acid-only option effectively discounts the information content of synonymous substitutions. Given the heterogeneity in numbers of both transitions and transversions at all three codon positions in the protein sequences reported here (Table 3), we think either weighting scheme is unrealistic.

Li et al. (1985a) developed a six-parameter model of protein molecular evolution to take into account additional possibilities of unequal substitution rates. This model estimates relative probabilities of nondegenerate (all possible changes are nonsynonymous), twofold degenerate (one of three changes is synonymous), and fourfold degenerate (all changes are synonymous) substitutions separately for each codon position. Such distinctions will incorporate transition/transversion biases, and the model assumes that these substitution rates are symmetrical.

The motivation for development of the six-parameter model derives from the observation that in simulation studies one- and two-parameter models underestimate true substitution rates under certain conditions. However, as Li et al. (1985b) pointed out, models with larger numbers of parameters would intuitively seem to be better descriptors of the processes being modeled than those known to be too simplistic. The character weights presented in the asymmetrical step matrix (Table 6) were derived from the inverse of the observed proportions of all 12 substitution possibilities. In other words, the weighting scheme is based on a 12-parameter model derived from the aligned sequences. If Li et al. are correct in their intuition, then the weights assigned in this matrix should be better than any of our other weighting options because they more accurately reflect true biases in mutation rates.

In terms of assessing the validity of our weighting scheme, the following computer simulation results are relevant (summarized by Li et al., 1985b). Models with different numbers of parameters show similar accuracy in estimating variable substitution rates when the total number of nucleotide sites sampled is modest (>140) and

when overall divergence (estimated as K, the mean number of substitutions per nucleotide site, between two sequences) is low ($K < 1.0$). When $K > 1.0$, the six-parameter models are inaccurate in a high proportion of cases, even when a large number of nucleotides (3,000) are sampled. We emphasize that sequence divergence in which $K > 1.0$ is typically characteristic of ordinal level divergence (in mammals), at least for the protein sequences used to derive the models. When divergence is lower than this and many bases are sampled, six-parameter models give the best estimates of substitution rates, and one-parameter models give the worst estimates. We cannot directly compare our frequency-based step matrices in Table 6 with the Li et al. (1985a, 1985b) analytical models but defend the choice of this weighting scheme on the basis of generally low overall sequence divergence between races (Tables 4, 5) and the large numbers of bases sampled.

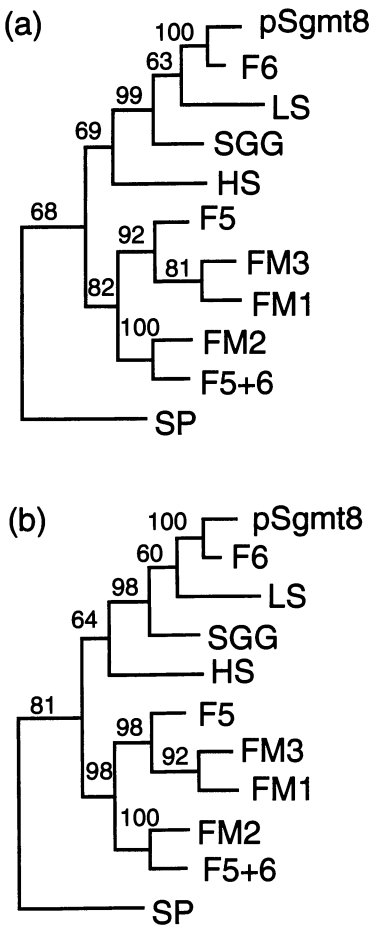

FIGURE 6. Bootstrap (100 replications) 50% majority rule consensus trees based on asymmetrically weighted base substitutions for all protein genes (Table 6) for *Sceloporus grammicus* ingroup taxa rooted with *S. poinsetti* (SP). (a) Tree obtained from asymmetric matrix of single input tree. (b) Tree obtained from asymmetric matrix of 100 randomly joined trees.

Chromosome Evolution in the S. grammicus Complex

Figure 7 presents our best-supported hypothesis for the history of establishment of the chromosomal rearrangements that define the *S. grammicus* complex. Here we have simply mapped known rearrangements onto the trees presented in Figure 6 in the most-parsimonious patterns. This interpretation differs from those previously presented in a number of ways. Hall originally hypothesized (1973, 1980) the sequence of derivation of the chromosome races of *S. grammicus* to be approximately linear in that they formed a series of successively higher $2n$ numbers originating via the sequential fixation of Robertsonian rearrangements (fissions) in different macrochromosomes (Fig. 1). He later referred to this as a cascade arrangement (Hall, 1983), but the conclusions were based entirely on Giemsa-stained karyotypes and therefore required independent testing. This model assumes strong underdominance for rearrangements in the heterozygous state and a single fixation for all rearrangements except either chromosome 5 or 6 (Figs. 1a, 1b).

Sites and Davis (1989) proposed a second phylogeny based on allozymes and the presence/absence of mapped restriction sites in both mtDNA and rDNA genomes. However, because of lack of many shared sites between the outgroup (*S. dugesii* from the *S. torquatus* group) and ingroup, not all the relationships were clearly resolved. Further, the F5 race had not been discov-

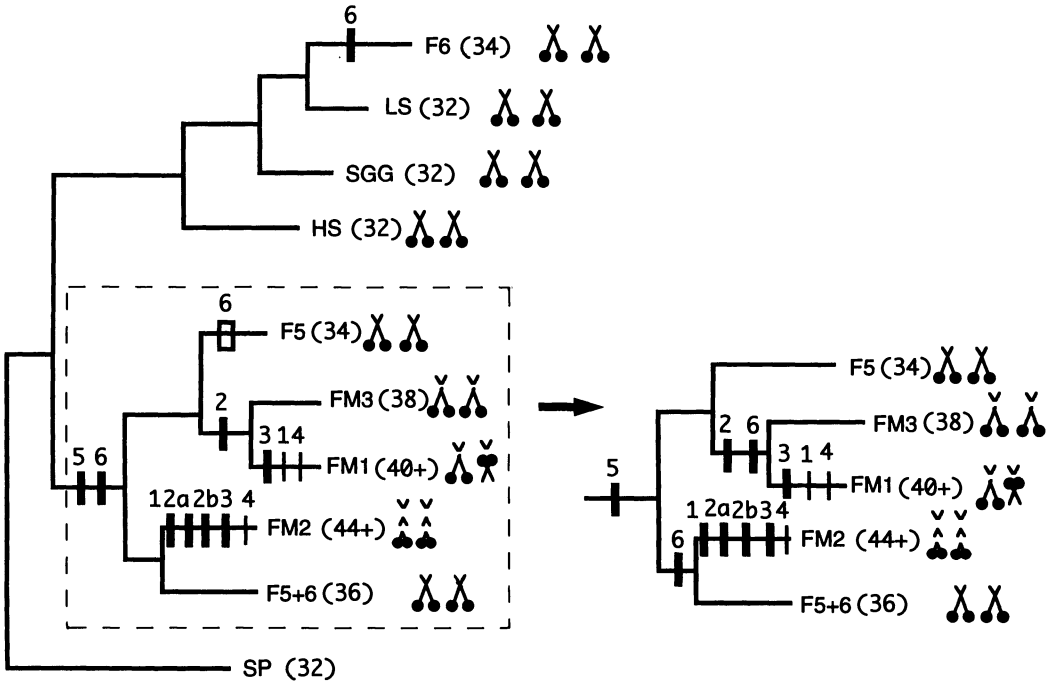

FIGURE 7. Most strongly supported phylogenetic hypothesis for the pattern of chromosome evolution in the *Sceloporus grammicus* complex (based on the topology presented in Fig. 6). The diploid numbers are given in parentheses for all taxa, as are morphologies for chromosome 2 (ingroup taxa only; solid circles show positions of NORs). Gains of chromosome fissions are indicated by solid rectangles (fixations) or vertical lines (polymorphisms), and fusions are indicated by open rectangles (fixation for chromosome 6 only). All rearrangements are mapped onto tree branches following the most-parsimonious interpretation for a given topology; alternatives are given for the high-2*n* clade, showing different histories for chromosome 6.

ered, and its absence is an important consideration because the omission of a single taxon may dramatically influence tree topologies under some conditions (Wheeler, 1992). In spite of these limitations, these workers showed that the multiple fission types (FM1 and especially FM2) appeared to be the most nested, and the HS race was basal to all others. Relationships among some other cytotypes were difficult to assess because of mtDNA introgression between some races and discrepancies between nuclear and mitochondrial markers. In this study, however, the incorrect recovery of hybridizing races as sister taxa due to extensive mtDNA introgression (Smith, 1992) is not an issue. With the exception of the LS × F6 hybrid zone, all other contacts occur between nonsister races, according to any of the topologies in Figures 5 and 6.

The hypothesis presented in Figure 7 suggests an alternative pathway of descent that contradicts the strictly linear cascade arrangement proposed by Hall (Fig. 1). The hypothesis in Figure 7 is strongly supported over both cascade alternatives in Figure 1 by the winning-sites test of Prager and Wilson (1988; 1a vs. best differ by 32 vs. 133 wins, respectively; 1b vs. best differ by 35 vs. 171 wins, respectively; $P < 0.001$ in both). The $2n = 32$ karyotype is supported as the plesiomorphic arrangement (six pairs of biarmed macrochromosomes), from which a fission of chromosome 6 was independently established at least twice, once in the F6 race within the low-2*n* clade and again (minimally) in the lineage to the high-2*n* clade. One interpretation is that the ancestral fission in the high-2*n* clade was then followed by a refusion of chromosome 6 in the lineage of the F5 cytotype

to reestablish the biarmed condition (left topology, Fig. 7). An alternative that avoids the refusion of chromosome 6 is possible but requires that two independent fissions must be fixed within the high-2*n* clade (right topology, Fig. 7). Cytogenetic mutational mechanisms are insufficiently understood to permit choosing between these two, but both require more changes in this chromosome than in all others, a situation that implies directionality in genome change due to nonrandom chromosomal rearrangement. Multiple origins of the same rearrangement overcome one of the most severe restrictions of many models of chromosomal speciation, the assumption of a single fixation event of a strongly underdominant rearrangement, either by sampling error alone or combined with inbreeding (Sites and Moritz, 1987).

Both cladograms in Figure 7 also present strong evidence for an independent origin of the chromosome 3 fission (once each in FM1 and FM2) and two or more origins for different chromosome 2 rearrangements. The same interpretation holds for the following macrochromosomal polymorphisms: (1) the fission of chromosome 1 arose once and became fixed in the FM2 race and arose again as a polymorphism in the FM1 race; (2) the fission of chromosome 4 similarly had two origins but has not become fixed in either the FM1 or FM2 races; and (3) chromosome 4 has undergone a third rearrangement as a pericentric inversion polymorphism in the F5 race (not depicted in Fig. 7). Alternative equally parsimonious hypotheses can be formulated for the origins of these same rearrangements if the trees produced by analyses of other data sets (e.g., those in Fig. 5) are used in place of those based on the asymmetrical mutation matrices, but the fundamental conclusion remains unaltered; chromosome evolution in these eight races from central Mexico has not been strictly linear in the sense proposed by Hall (Fig. 1). Given the geographically widespread occurrence of Robertsonian (fission and fusion) and pericentric inversion polymorphisms known from other parts of the range (Sites, 1983) and the disjunct distributions of what superficially appear to be some of the same races outside of central Mexico (especially LS, F5, and F6; Sites et al., 1987: fig. 4), it is not surprising that multiple, independently established rearrangements are common in the central Mexico races. The previously dominant view that a novel chromosomal rearrangement has a very low probability of fixation loses force if the same rearrangement is generated repeatedly, especially if the rearrangement is not substantially underdominant in its meiotic effects (Patton and Sherwood, 1983; Sites and Moritz, 1987). Such findings allow for the fixation of chromosomal rearrangements for adaptive reasons, and some workers are beginning to look for phenotypic effects of chromosomal rearrangements that may have important fitness consequences (Shaw et al., 1988; Groeters and Shaw, 1992).

The Minimum-interaction Hypothesis

Imai et al. (1986) proposed that a primary deterministic force governing chromosomal evolution in eukaryotes has been selection for reduced opportunity for spontaneous negatively heterotic chromosomal mutations. The majority of spontaneous rearrangements occur in synaptonemal complexes via crossovers and subsequent misresolution of interlockings between elements during pachynema, a stage of meiotic prophase in which bivalents are extremely elongated and fixed at their telomeres to the nuclear membrane. This arrangement of bivalents in prophase nuclei is highly structured and nonrandom and represents a universal configuration in eukaryotes referred to as the suspension-arch structure by Imai et al. (1986). Because potentially deleterious rearrangements such as reciprocal translocations result from interactions between nonhomologously associated chromosomes, the configuration of bivalents significantly affects the occurrence probabilities of these kinds of rearrangements. Specifically, the size of the autosomes and nuclear volume interact to determine the configuration of the suspension-arch structure and therefore the frequency with which different combinations of bivalents may interact.

The model developed by Imai et al. sug-

gests that selection should act to reduce bivalent interaction probabilities leading to reciprocal translocations by acting on the chromosome size ($2n$)/nuclear volume (r) ratios of oocytes and/or spermatocytes at pachytene. This hypothesis predicts that when the $2n/r$ ratio is low, the frequency of reciprocal translocations should be low and an increase in diploid number by centric fissioning should not be strongly selected for. Conversely, under high $2n/r$ ratios, selection should favor the establishment of fissions to reduce the sizes of the largest autosomes, thereby reducing their interaction probabilities. Evidence from meiotic pairing behavior in some *S. grammicus* cytotypes suggests that reciprocal translocations (and other complex rearrangements) can occur in the macrochromosomes (Porter and Sites, 1986: fig. 7; Arévalo et al., 1991: figs. 8, 9), but the order of fissioning of these elements does not conform to the prediction of the minimum-interaction hypothesis (Fig. 7). The smallest macrochromosomes, pairs 5 and 6, are the first to fission, whereas the intermediate-sized (3 and 4) and largest (1 and 2) elements are the most recently derived in the complex (Fig. 7). The present study is the first phylogenetic test of the minimum-interaction hypothesis, and its failure to predict the correct sequence of chromosomal fissions in the *S. grammicus* complex may have general significance for mechanisms of eukaryote genome evolution if these observations can be confirmed in other groups.

The Evolution of Chromosome 2

Reed et al. (1992c) were able to clarify some unresolved problems in the FM2 cytotype, using microspreading and synaptonemal complex techniques to examine the earliest stages of meiotic pairing. These workers showed that the nucleolar organizer region (NOR) was present on the telomeric end of a medium-sized acrocentric chromosome in FM2 in contrast with its usual location on the telomere of the long arm of the biarmed chromosome 2 (in LS, HS, F5, F6, and F5+6) or the large acrocentric fission product of chromosome 2 in FM3 (F2+5+6) and FM1 (see Fig. 7). (The FM1 race is polymorphic for NOR position; it may be either at the centromeric or telomeric end of the large acrocentric chromosome 2 [Reed et al., 1992c: fig. 7] .) The corresponding size and number of chromosomes between the F5 and FM2 races examined by Reed et al. suggest that chromosome 2 has undergone multiple rearrangements to derive the morphology in FM2.

When these different chromosome 2 morphologies are placed onto the cladograms in Figure 7, the evolutionary history of the NOR position can be inferred. The NOR position on chromosome 2 in the FM3 race is likely the result of a simple Robertsonian fission of this chromosome, either as a synapomorphy with FM1 (either topology in Fig. 7) or independent of the FM1 race (other cladograms in Fig. 5), whereas the NOR polymorphism in FM1 is presumably further derived by a pericentric inversion of the acrocentric NOR-bearing element. Independent of these events, the derivation of the FM2 morphology of chromosome 2 appears to have resulted from the fixation of the two different rearrangements in the original submetacentric state. One of these was a centric fission (similar to the rearrangement characteristic of FM3 and FM1), and another rearrangement was necessary to produce two small acrocentric elements (one bearing the NOR) characteristic of FM2 (labeled 2a and 2b in Fig. 7). Derivation of this second rearrangement is interesting, because an interpretation of a second fission in the longer arm may require a mechanism not previously widely considered. This second fission could occur by the sequence of rearrangements hypothesized by Reed et al. (1992c): centric fission → pericentric inversion in the long arm of chromosome 2 → second centric fission, which would transform chromosome 2 from a single large submetacentric element to three smaller acrocentric elements characteristic of the FM2 race.

A more interesting possibility is that a previously quiescent centromere in the long arm of chromosome 2 was activated

and then provided the molecular structure needed for a second fission event. Because both chromosome 2 rearrangements appear as autapomorphies in the FM2 race, the exact sequence of events in the derivation of its unique morphology cannot be determined from the cladogram alone. The possibility of a latent centromere in chromosome 2 must await confirmation at the molecular level of chromosome structure, but in view of documentation of such structures in other groups (Earnshaw and Midgeon, 1985; Merry et al., 1985), detailed molecular studies of chromosome 2 in the *S. grammicus* complex would certainly be worthwhile.

Hybrid Zones and Hybridization in the S. grammicus Complex

Figure 7 shows that the LS × F6 contact is the only one known to occur between sister groups. This contact is known from lower elevations on mountain ranges surrounding the Valley of Mexico, whereas at higher elevations on these same slopes, the F6 cytotype forms a narrow hybrid zone at the upper elevational limit of its distribution with the HS cytotype (Hall and Selander, 1973; Arévalo et al., 1993). Thus populations involved in both the LS × F6 and F6 × HS contacts are distinguished by a single fission at chromosome 6 but would be expected to differ substantially in their overall genetic divergence on the basis of their phylogenetic relationships (Fig. 7). This expectation has been confirmed by both isozyme and restriction mapping studies (Sites et al., 1988a; Sites and Davis, 1989) showing that the HS race is very distinct from F6 and LS, and populations of HS isolated on different mountain peaks can be consistently recovered as a monophyletic group on the basis of multiple nuclear and mitochondrial markers (Sites and Davis, 1989). In contrast, the LS race is paraphyletic with respect to F6 for mtDNA (Sites and Davis, 1989), and introgression of single-copy nuclear gene markers and mtDNA haplotypes is substantial across the LS × F6 contact relative to the extremely limited introgression for either between F6 and HS (Arévalo et al., 1993). These observations suggest that the degree of overall genetic divergence between hybridizing populations is more important than a single chromosomal rearrangement in restricting gene flow. Single rearrangements, therefore, appear to contribute little to hybrid unfitness when genetic divergence between hybridizing populations is low, and this result contradicts the single most important assumption of some models of chromosomal speciation (Sites and Moritz, 1987).

Further testing of the relationships of the *S. grammicus* chromosome races proposed here should be carried out with nuclear gene sequences, and hybrid zone interactions should be studied in depth between closely and distantly related races differing by multiple rearrangements.

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