

# Chromosome variation, genomics, speciation and evolution in *Sceloporus* lizards

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# Chromosome variation, genomics, speciation and evolution in *Sceloporus* lizards

**Abstract.** The clade of the North American lizard genus *Sceloporus* and its relatives comprising the subfamily Phrynosomatinae (Iguanidae) include perhaps 150 evolutionary lineages. The work reviewed here begins with the discovery more than 40 years ago of the concentration of Robertsonian chromosomal variability in *Sceloporus* and cytogenetic and genomic evidence of remarkable chromosomal variation within the *S. grammicus* complex associated with narrow zones of hybridization between different chromosomal races. These discoveries led to hypotheses about hybrid zones involving negative heterosis, possible modes of chromosomal speciation, and the potential roles of such

speciation in phylogenesis. The radiation of *Sceloporus* has now been studied by many different workers extending and mapping the geographic distribution of cytogenetic and genomic variation to understand the biology of the chromosomal variation and to establish the phyletic relationships of the various lineages. The result is a robust phylogeny and a large and still growing database of genic, cytogenetic and other biological parameters. These materials provide a rich series of natural experiments to support both synthetic-comparative and analytical studies of the roles of chromosomal variation, hybrid zones and modes of speciation in phylogenesis and evolutionary success.

In the mid 1960's, when I began graduate research, *Sceloporus* (Iguanidae<sup>[1]</sup>) was known to have an exceptional number of species, ecological diversity, and geographic range (Smith 1939; Sites et al. 1992). Smith and Taylor (1950) recognized 54 species of *Sceloporus*, ranging from Canada to Panama, and around 50 *Anolis* (Iguanidae) in North America. The next most speciose lizard genera were *Eumeces* (Scincidae) with 22 species, and *Cnemidophorus* (Teiidae) with about 17 species. More recently, Uetz (2009) lists 88 *Sceloporus*. In the Iguanidae, only the tropical *Anolis* radiation of some 369 species in North and South America plus the West Indies (Poe 2004) and the *Liolaemus* radiation of at least 200 species (Avila et al. 2008, Pincheira-Donoso and Nunez 2008; Pincheira-Donoso et al., 2008) in southern South America have more species than *Sceloporus*.

Aside from the number of species, *Sceloporus* are ecologically diverse, ranging from below sea level in extreme deserts to above the timberline and to tropical rain forests. Sympatry is common, with three or more species occurring syntopically over most of Mexico and the SW United States. It also seemed that *Sceloporus* is recently evolved compared to related genera, as confirmed in later works (Schulte et al. 2003; Wiens et al. 2009).

Important evolutionary questions implicit in this list of superlatives were: Why and how has *Sceloporus* evolved so many more species than have other genera of related and older origins, and might understanding these questions provide a more general insight into problems of species formation and adaptive radiation? Although the speciation problem could not be subjected to

controlled experiments in the laboratory, subdivisions within *Sceloporus* - and the existence of several other closely related genera in the sceloporine - now known as the phrynosomatine<sup>[1]</sup> branch of the family (Savage, 1958; Etheridge, 1964; Schulte et al. 2003) - provide natural experiments (Diamond 1986) that might be studied by the comparative approach to determine possible relationships between various biological characteristics of the subdivisions and their differing evolutionary successes (Hall 1973, 1983).

The first indications that *Sceloporus* showed exceptional chromosomal variability (Hall 1965) compared to other iguanid genera and especially closely related ones, suggested that chromosomal changes might facilitate speciation (Hall 1965, 1973). As a budding evolutionary biologist, I was interested in the possible roles of genetic systems in evolution, where the genetic system consists of all aspects of a species biology affecting the transmission of its heredity from one generation to the next (Darlington 1958; Stebbins 1950; Mayr 1963; Lewis and John 1963) that are themselves subject to selective pressures and evolution. This specifically includes cytogenetics and genetically mediated aspects of population structure and mate choice. The comparative approach (Hall 1983; Harvey and Pagel 1991; Miles and Dunham, 1993) is used here to explore these questions across the wider data set now available.

## Chromosomal conservatism and variability

By the time I begin serious work on *Sceloporus* cytogenetics, parallel studies were already under way by C.J. Cole and his associates at University of Arizona (Cole et al. 1967; Lowe et al. 1967; Cole, Lowe 1968 – see Sites et al. 1992 for additional references to Coles' early work). Our respective studies confirmed the concentration of chromosomal variability in *Sceloporus* and encouraged Cole and myself to continue gathering evidence. One of the first uses of karyological evidence was to support speculations regarding the distribution and directions of chromosomal changes in *Sceloporus* and how this could be used to understand phylogenetic relationships within the genus.

### Karyotypic conservatism

It was clear that some iguanids were chromosomally conservative (Gorman 1973). Several clades included species with a karyotypic pattern of  $2n=36$  with 6 pairs of metacentric and submetacentric macrochromosomes, often with a secondary constriction (nucleolar organizing region) on the long arm of the largest submetacentric chromosome, 12 pairs of microchromosomes, and no distinguishable sex chromosomes (Paull et al. 1976 and Fig. 1). Gorman (1973) noted that this pattern had also been found in the Chamaeleontidae, Gerrhosauridae, Anguidae, Amphisbaenidae and Agamidae. By any account, the retention of this apparently ancestral karyotype by living species across such a wide range of families, where some of the family-level bifurcations appear to trace back at least to cladistic separations before the Jurassic breakup of Gondwanaland (Moody 1980; Macey et al. 1997; Conrad et al. 2007; Okajima and Kumazawa 2008), suggests a remarkable degree of chromosomal conservatism in these lineages.

### Interspecific karyotypic variability

Sites et al. (1992) tabulated all extant chromosomal information for *Sceloporus*, including otherwise unpublished work by Cole and myself, and provides the comprehensively authoritative reference for its karyotypic<sup>[2]</sup> variability. Excepting some *Sceloporus*, all karyotyped species within each phrynosomatine genus have a  $2n=34$  karyotype that differs from the common iguanid pattern only by having one less pair of microchromosomes, and including an x and y sex chromosome pair among the microchromosomal complement of 22 pairs. Although Lowe et al. (1967) and Cole (1970, 1971) argued otherwise, given its similarity to the primitive iguanid pattern, this  $2n=34, xx♀, xy♂$  is assumed to be primitive for phrynosomatine lizards including *Sceloporus*. Within *Sceloporus*, the primitive karyotype is also found in the majority of the species groups recognized by Smith (1939; Flores-Villela. et al. 2000; Sites et al. 1992; Wiens 1993).

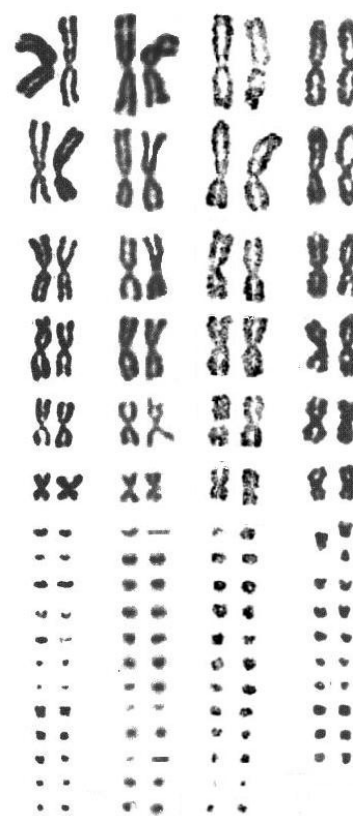


Fig. 1. Comparison of “primitive” karyotypes. From left to right; *Stellio caucasica*  $2n=36$  (from Hall 1973), *Liolepus belliana*  $2n=36$  (Hall 1970) both Agamidae; *Conolophus subcristatus*  $2n=36$  (an early branch of the iguanid subfamily Iguaninae from the Galapagos Islands - Paull et al. 1976) and a male *Sceloporus grammicus* “standard”  $2n♀=32, X_1X_1, X_2X_2$ ,  $2n♂=31, X_1, X_2, Y$  (Hall 1973). By contrast to the other phrynosomatine genera, extensive chromosomal variability occurs in several clades in *Sceloporus*, both between and within the current taxonomic species as summarized in Hall (1977) and Sites et al (1982). Samples of this variability are illustrated in Fig. 2 and Fig. 3.

## Taxonomy, phylogeny, and the sequence of chromosomal derivation

### Cladogenesis

Comparative analyses to understand the chromosomal variability in *Sceloporus* depend critically on a well grounded phylogeny (Hall 1983; Harvey and Pagel 1991; Miles and Dunham, 1993). Ideally, any phylogeny used for a comparative analysis of the roles of chromosome variation in evolutionary processes should be based on data independent from chromosomal changes. Early phylogenetic hypotheses for the derivation of the phrynosomatine genera by Savage (1958), Etheridge (1964), Presch (1969) were based on morphology and seemed to provide clear sister clades for comparison with the chromosomally variable *Sceloporus* (Fig. 6). Smith (1939) and Larsen and Tanner (Larsen 1973; Larsen and Tanner 1974, 1975) offered such phylogenies for *Sceloporus* species groups and species. However, both were problematic.

With some modifications, Smith's (1939) species groups are still useful. However, his phylogenetic reconstruction was based on the idea that *Sceloporus* emerged from South America via the *formosus* group.

This reconstruction was refuted by karyotypic data showing that *formosus* group species all had highly derived karyotypes compared to the conservative  $2n=34$  karyotype (Hall 1973, 1977). All subsequent phyletic studies based at least in part on non-chromosomal data have also shown *formosus* group species to be highly apomorphic rather than basal (see Larsen and Tanner loc. cit., Sites et al. 1992, Reeder and Wiens 1996, Wiens and Reeder 1997, Flores et al 2000, Smith 2001, Leaché 2009, Wiens et al. 2009).

Larsen and Tanner's phylogeny (Larsen 1973; Larsen and Tanner 1974, 1975) was based on a multivariate phenetic analysis of 40 characters of external morphology and 40 skull characters. Only 5 of the 80 characters are independent of skull and scalation. Hall (1973) and Sites et al. (1992) both noted similar problems with the characters chosen and their usage that need to be considered carefully in any phenetically based multivariate study. In *Sceloporus*, many skull characters are probably highly adaptive in covariant ways to prey capture and feeding (Meyers 2002).

Similarly, scalation (i.e., size, robustness and sharpness of individual scales) defends against predation and the environment (Smith 2001). Most of Larsen's characters are based on measurements expressed in ratios to reduce dependence on the absolute size of the organisms measured. On the other hand, many such measures in iguanid lizards show important allometric changes with growth or size (de Queiroz 1987). Where this occurs, the allometry may apply to related species differing in size, reducing supposedly independent ratios back to the one variable – body size. Beyond these problems, *Sceloporus* vary in dorso-ventral flattening depending adaptations to use shimmy burrowing/sand diving, dug burrows, wood crevices, or rock crevices for escape cover (Hall 1973). Many morphological characters will co-vary with adaptation to this single habitat choice and again it seems possible that any multivariate phenetic approach will give undue weight to what boil down to a small number of adaptive choices. Karyological and genomic characters are likely to provide better clues for cladistics.

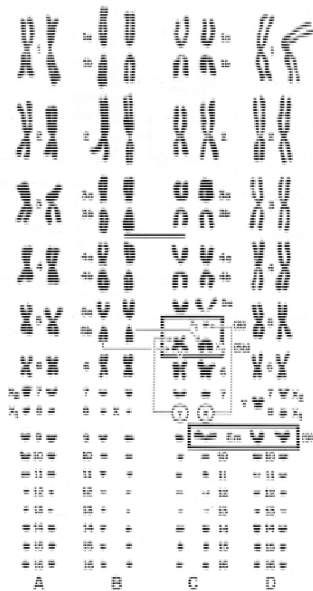


Fig. 2. Karyotypic relationships of the *clarki* group species (from Hall 1973, 1977). **A.** Standard crevice user karyotype, represented by *S. mucronatus* ♀. **B.** *S. clarki* ♀ karyotype. **C.** *S. melanorhinus* ♂ karyotype, heterozygous for the Em chromosome. **D.** *S. asper* ♂ karyotype, fixed for the Em chromosome. Em chromosomes and the sex chromosomes of *melanorhinus* are enclosed in boxes. The long arm of the *melanorhinus* neo-Y probably derives from one of the acrocentric fission products of the standard pair 5, which is fused to the "y" of *clarki* – which in turn probably corresponds to the long arm of the "y," chromosome of the standard-crevice user.



Fig. 3. Male diakinesis arrays for species deriving from the extinct,  $2n=32$  polymorphic Em ancestor (from Hall 1973, 1977). **A.** *S. torquatus*, **B.** *S. grammicus* "S", **C.** *S. clarki*, **D.** *S. melanorhinus*, **E.** *S. m. megalepidurus*, **F.** *S. asper*. Note the sex chromosome heteromorphism of all species. Also note the heteromorphous pairing of the chromosomes in the 9th bivalent of *melanorhinus* (column D) due to heterozygosity for the Em chromosome. Chiasma positions in the sex chromosomal bivalents or trivalents are indicated by small bars.

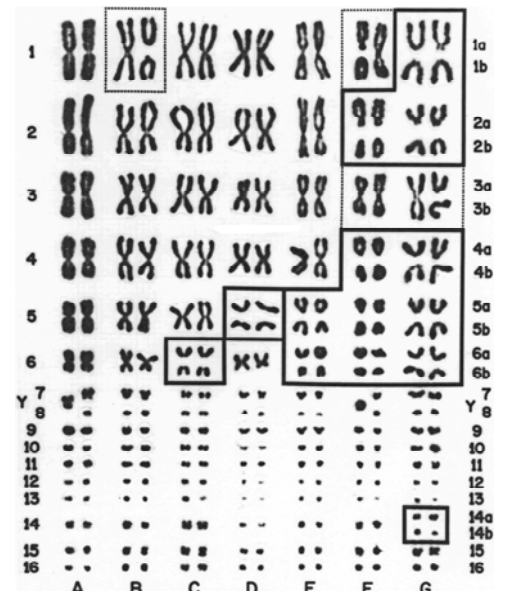


Fig. 4. Major karyotypes of the *grammicus* complex. (from Hall 1973, 1977) **A.** standard *grammicus* ♂; **B.** P1 *grammicus* ♀; **C.** F6 *grammicus* ♀; **D.** F5 *grammicus* ♀; **E.** F5+6 *grammicus* ♀; **F.** FM1 *grammicus* ♂; **G.** FM2 *grammicus* ♀. Hall and Selander (1973 Figs. 2 and 3a) map the distribution of each karyotype pattern shown here. Chromosomes which differ from the primitive or "standard" condition for all crevice-users are enclosed in boxes. If the condition is fixed, the border is solid; and if it is polymorphic, the border is dashed. Note: Sites has termed the "standard" race, LS (for "low standard"), and the P1 race HS (for "high standard")



Fig. 5. **A.** Mitosis and **B.** two diakinesis spreads from adjacent cells in *S. occidentalis* ♂ (MCZ R110098 – *undulatus* group) showing centromeric location of chiasmata (Hall unpublished data).

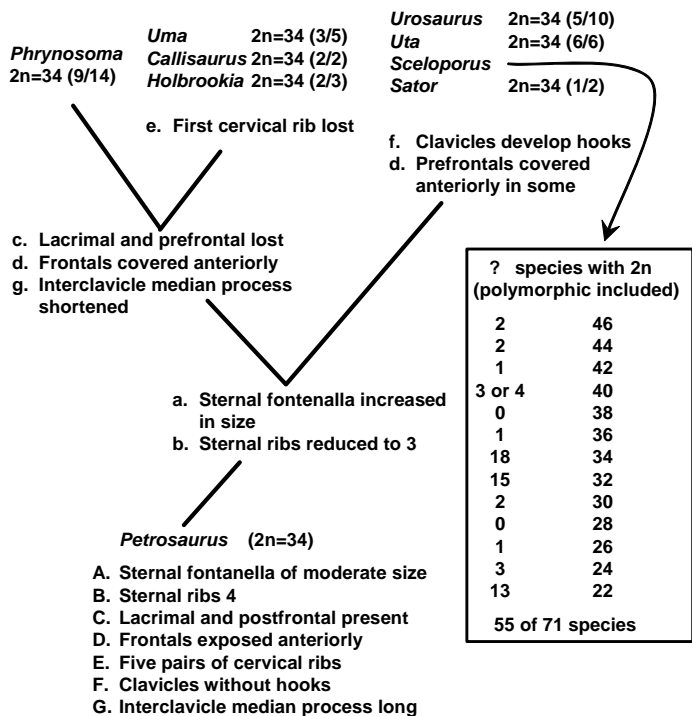


Fig. 6. Karyotype distribution in the sceloporines/Phrynosomatidae (diagram based on Presch 1969, from Hall 1973). *Petrosaurus* is the sister genus to the sceloporine genera. Ratios in parentheses are the number of karyotyped species known to me in 1973 for each genus over the total species in each genus.

In 1973 my doubts about Larsen and Tanner’s phenetically derived phylogeny were greater than concerns about using chromosomal data to aid reconstructing the derivations of the species groups. The assumption was that chromosome mutations were rare and that the 2n=34 pattern found in all the likely sister genera to *Sceloporus* was plesiomorphic (Fig. 6). Thus, apomorphies could be clearly identified for constructing some important cladistic branching points.

*Sequence of chromosomal derivation*

My early interpretation (Hall 1973, 1977) of the cladistic distribution of chromosomal variation<sup>[3]</sup> across species groups in *Sceloporus* is summarized in Fig. 7, Fig. 8, and Fig. 9. Although definitions of particular species groups have varied, subsequent studies increasingly based on genomic data largely concur with the reconstruction shown here (Hall 1977). The main

changes for basal groups (Fig. 7) are that *couchi* should now be put in the *variabilis* group, and that *Sator*’s (Fig. 6) species are now included within *Sceloporus* alongside *utiformis* (Sites et al. 1992, Wiens 1993, Reeder and Wiens 1996, Wiens and Reeder 1997, Flores-Villela et al 2000, Smith 2001, Leaché and Mulcahy 2007, Leaché 2009; Wiens et al. 2009).

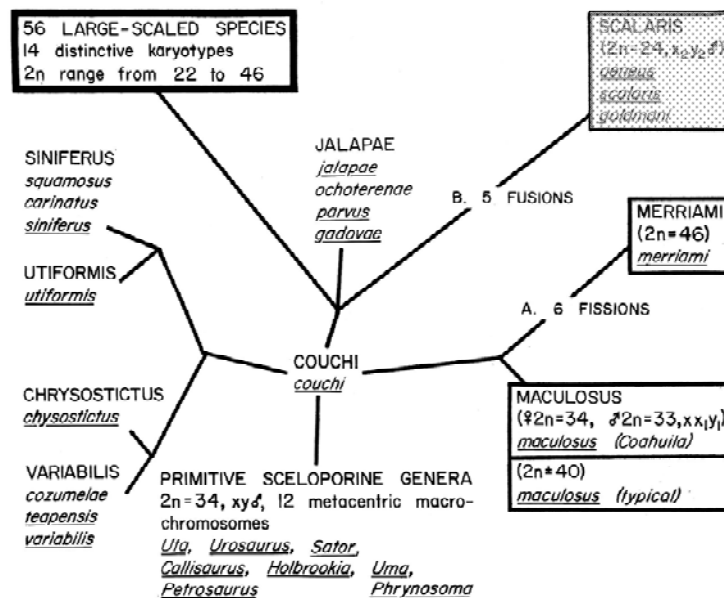


Fig. 7. Species groups and postulated sequences of derivation in the basal *Sceloporus*, more or less corresponding to Smith’s (1939) “small-scaled, small-sized” division (from Hall 1977). Underlined names indicate genera or species for which karyotypic information was available through 1976. Move *scalaris* to Fig. 9.

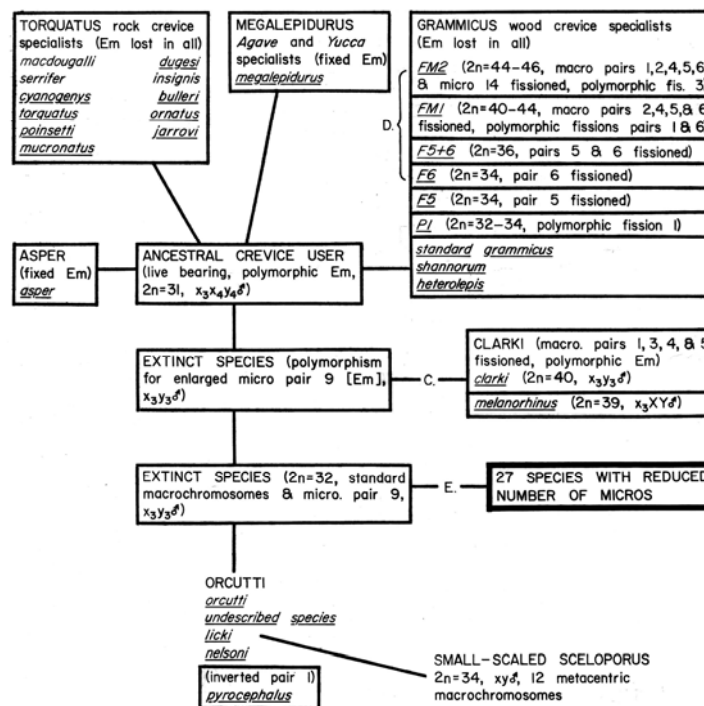


Fig. 8. Species groups and postulated sequences of derivation in the generally large scaled *Sceloporus*, more-or-less corresponding to Smith’s (1939) “large-scaled, large-sized” division (from Hall 1977)

The major surprise from genomic phylogenies relates to placement of the 2n=24 *scalaris* group (Fig. 7), differing from the plesiomorphic 2n=34 by the “loss” (presumably by fusion) of 5 pairs of micro-

chromosomes. In placing *scalaris* in the small-scaled, small-sized division, Smith (1939) and Hall (1977) gave precedence to its similar size and appearance to small-scaled, small-sized species. However, Leaché's (2009) combined nuclear genomic data convincingly places the branch to the *scalaris* group as sister to the generally large-scaled, large-sized species groups with 2n=22 karyotypes, as does the Wiens et al. (2009: Figure 5) tree from maximum likelihood analysis of combined nuclear and mitochondrial DNA data from 122 taxa. Wiens et al's other trees show substantial variations in placing the chromosomally derived large-scaled species. Flores-Villela et. al (2000) also place *scalaris* at intermediate branching levels among the large sized, large scaled radiation.

It is now clear that the *scalaris* branch should be removed from Fig. 7 to Fig. 9 as a branch off the extinct species (2n=24) between *magister* (2n=26) and *horridus* (2n=22) – where both chromosome and genomic data independently place it. Morphologically and ecologically, *scalaris* are not strikingly different from some of the smaller members of the *horridus/undulatus* group such as *S. virgatus* (treated as a subspecies of *undulatus* in Hall 1977).

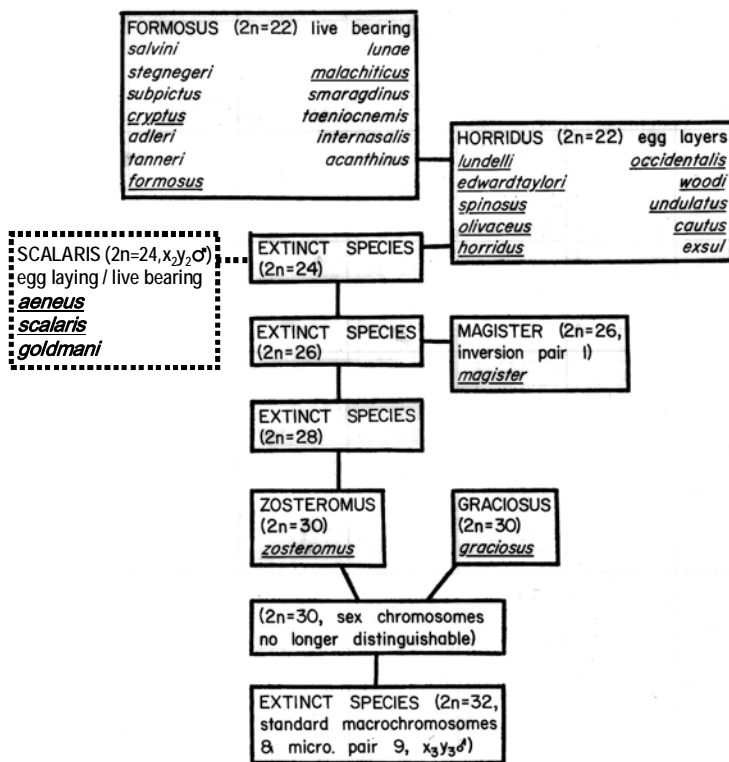


Fig. 9. Species groups and postulated sequence of chromosomal variation leading to further reduction in the number of microchromosomes. Note: the "horridus" group as defined by Hall (1977) and followed by Sites et al. (1992) combines Smith's (1939) *spinosus* and *undulatus* groups as per (Bell et al 2003). Following the rule that the oldest available species name should be applied to the group as a whole, this should have been called the "undulatus" group. Hall used the name *horridus* to avoid confusion with either of the group names as constituted by Smith (1939). The *scalaris* group has been moved here from Fig. 7.

Thus, there appear to be two completely independent sequences of karyotypic derivation in *Sceloporus*; one involving six macrochromosomal fissions leading to *S.*

*merriami* (Fig. 7) – an early branch in the radiation of *Sceloporus*, adapted to a geographically compact association with bunch grasses at high elevations, and a complex branching sequence involving most of Smith's (1939) large-scaled, large-sized species (see Hall 1973, Sites et al 1992 for geographic distribution maps).

The latter radiation (Fig. 8, Fig. 9) includes a sequence leading to a reduction in microchromosome numbers from 11 pairs to 5 pairs<sup>[3]</sup>, possibly involving microchromosomal fusions; and a sequence of fissions of all the macrochromosomes (at least as polymorphisms) in the *grammicus* complex (Hall, 1973). Several chromosomally distinctive side branches survive, as shown in these Figs. The 2n=34 basal members of this radiation show parapatric and disjunct distributions around the Gulf of California separated across possible past seaways in the Baja California Peninsula, and across the Rio Grande Santiago – the major western drain from the Mexican Plateau. Most species deriving from a 2n=32 ancestor (Fig. 8) are predominantly users of trees and rocks along the eastern coast of the Gulf of California or belong to the widely distributed and speciose *torquatus* group of rock crevice users and *grammicus* group of wood crevice users. *S. zosteromus* (Fig. 9) and *rufidorsum* (not shown) are 2n=30 species that are ecologically dominant in the arid areas of Baja California (Grismer and McGuire 1996; Leaché and Mulcahy 2007), while the 2n=30 *graciosus* occupies high altitudes on the northwestern margins of *Sceloporus* distribution, with the related and more specialized *arenicolus* occupying sandy areas along the Texas, New Mexico border (Chan et al. 2009). The 2n=26 *S. magister* complex dominates the North American deserts exclusive of the areas of Baja California occupied by *zosteromus* and *rufidorsum* (Grismer and McGuire 1996; Leaché and Mulcahy 2007). Based on geographically sparse sampling of mitochondrial genes Schulte et al. (2006) recognized three species in the 2n=26 complex and confirmed the separation of these species from the 2n=30 forms in Baja California. Leaché and Mulcahy (2007), with much denser geographic sampling and nuclear as well as mitochondrial genes did not accept Schulte et al's splitting of species in the 2n=26 *magister*. The 2n=22 *horridus/undulatus* and *formosus* groups dominate *Sceloporus*' distribution over North and Central America exclusive of Baja California, the Sonoran Desert and the far northwest USA.

A possibly interesting situation worth further research is based on an unpublished observation by Carol Axtell that a single *maculosus* collected from near the species' type locality had a 2n around 40 (see Hall 1977). A possibility is that that the 2n≈40 individual represents intraspecific variation in *maculosus* itself. Alternatively, the rarely studied *maculosus* may not be monotypic, and the 2n≈40 *maculosus* individual may be an intermediate species leading to *merriami*, while the other karyotyped individuals belong to an unrecognized species. *S. maculosus* is treated as immediately apomorphic to the

$2n=46$  *merriami* group by Wiens and Reeder (1997) and reasonably close in the branching sequence before the emergence of the large-sized, large scaled species by both Flores-Villela et al (2000). However, none of the recent trees of Leache (2009) or Wiens et al. (2009) support this possible relationship.

### Karyotypic variation and hybridization in the *S. grammicus* complex

#### Early work

Hall and Selander (1973) and Hall (1973) reported a remarkable pattern of geographically distributed chromosomal variability within what had been considered to be a single species, *Sceloporus grammicus*, due to macrochromosomal fissioning. Karyotypes in various populations (Fig. 4) ranged from a  $2n_{\text{♀}}=32(x_1x_1,x_2x_2)$ ,  $2n_{\text{♂}}=31(x_1,x_2,y')$  with 6 pairs of metacentric macrochromosomes, to populations with 12 pairs of acrocentric macrochromosomes, where all macrochromosomes have fissioned, at least as polymorphisms. Based on their geographic distributions, at least five different chromosomal races could be distinguished (Fig. 10). Narrow parapatric contact zones a few hundred meters wide were found in the Valley of Mexico (Fig. 11a) for two combinations of races, F6 (Fig. 4c) × P1/HS (Fig. 4b) on the mountains east of Mexico City near Rio Frio (Fig. 11b – Hall and Selander 1973) and Standard/LS (Fig. 4a) × FM2 (Fig. 4g) along and near the Street of the Dead in the ancient city of Teotihuacán (Fig. 11a - Hall 1973). Based on the presence of individuals with mixed karyotypes, both pairs of chromosomal races hybridize where they meet.

P1/HS and F6 differed by one fixed chromosomal difference (fission of chromosome 6 in F6) and two fixed isozyme differences. Based on the recovery of F<sub>1</sub> and backcross individuals as indicated by heterozygosity for markers, the two chromosomal races met in a narrow hybrid zone no more than 400 m wide between “pure” populations on either side. Despite the plausibility that a single individual might disperse a few hundred meters during its life-time and the apparently free hybridization where the two populations meet, both populations from a few hundred meters away from their zones were genetically more similar to a population on the opposite side of the Valley of Mexico and to a small disjunct population of F6 on the Nevado de Colima, approximately 500 km to the west, than they were to each other. The inescapable conclusion is that the two adjacent populations are genetically isolated despite their hybridization.

A similar but less well substantiated hybrid zone (lacking isozyme data) was found where Standard/HS and FM2 populations meet in the Valley of San Juan Teotihuacán (Fig. 11a). These populations differed by four and possibly as many as 6 chromosomal differences (fissions of macrochromosomes 1, 2, 4, 5, 6 and a microautosome). The two races hybridize in the

Teotihuacán Archeological Zone, and are not completely sterile as determined by the recovery of 8 presumptive F<sub>1</sub> hybrids and 4 to possibly as many as 8 presumptive backcrosses (Hall 1973). Again, the width of the zone in which hybrids were found is at most a few hundred meters wide, with little evidence of introgression outside of it.

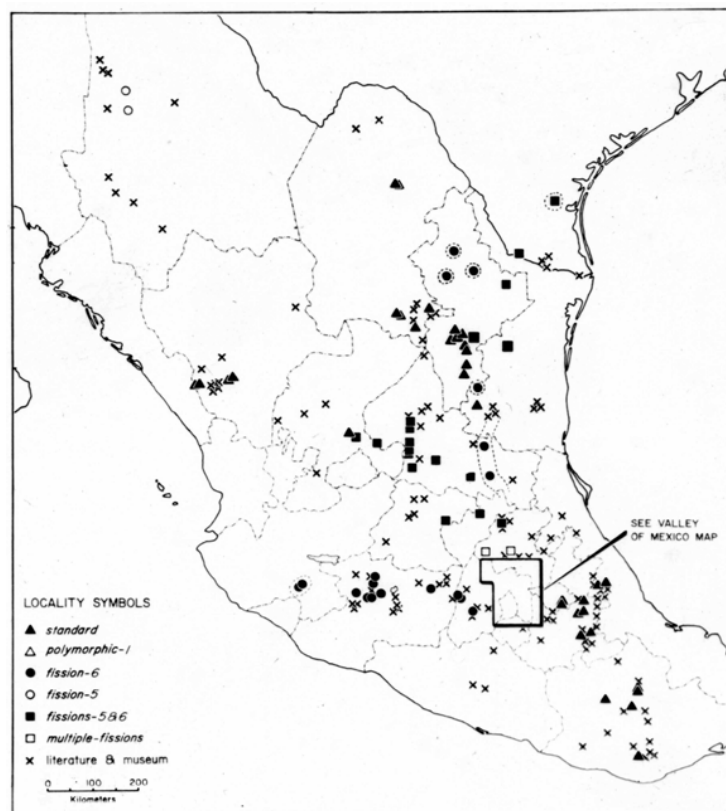


Fig. 10. Distribution of the karyotypically distinctive variants of *Sceloporus grammicus* in Mexico (from Hall 1973, Hall and Selander 1973). More recent maps with additional data are provided by Sites (1983) and Arévalo et al (1991). Note: the Sites group (e.g., Sites 1983, Arévalo et al 1991), refer to the “standard” race as “low standard” or “LS” because it is primarily found at low elevations in the Valley of Mexico. “Polymorphic-1 or P1 is termed “high standard” or “HS” because of its genetic similarity to LS and because it is primarily found at high elevations.

#### Subsequent studies of the *grammicus* complex

The distribution of the chromosomal races, their genetic variability, phyletic relationships, and possible statuses as evolutionary species has been followed up by Sites (1980, 1982, 1983), Sites and Dixon (1981), Sites et al. (1988), Sites and Davis (1989), Porter and Sites (1986), Thompson and Sites (1986), Arévalo et al. (1991; 1994), and Marshall et al. (2006). Population cytogenetics has been studied by Arévalo et al. (1991), Goyenechea et al. (1996), Porter and Sites (1985, 1986, 1987), and Sites et al (1988a). The genetic structures of additional hybrid zones to those described in Hall and Selander (1973) and Hall (1973) (Fig. 11) are reported by Arévalo et al. (1993), Degnan and Arévalo (2004), Dosselman et al. (1998), Marshall and Sites (2001), Reed et al (1995, 1995a), Reed and Sites (1995), and Sites et al. (1993, 1995, 1996). The following tentative conclusions can be drawn from these data.

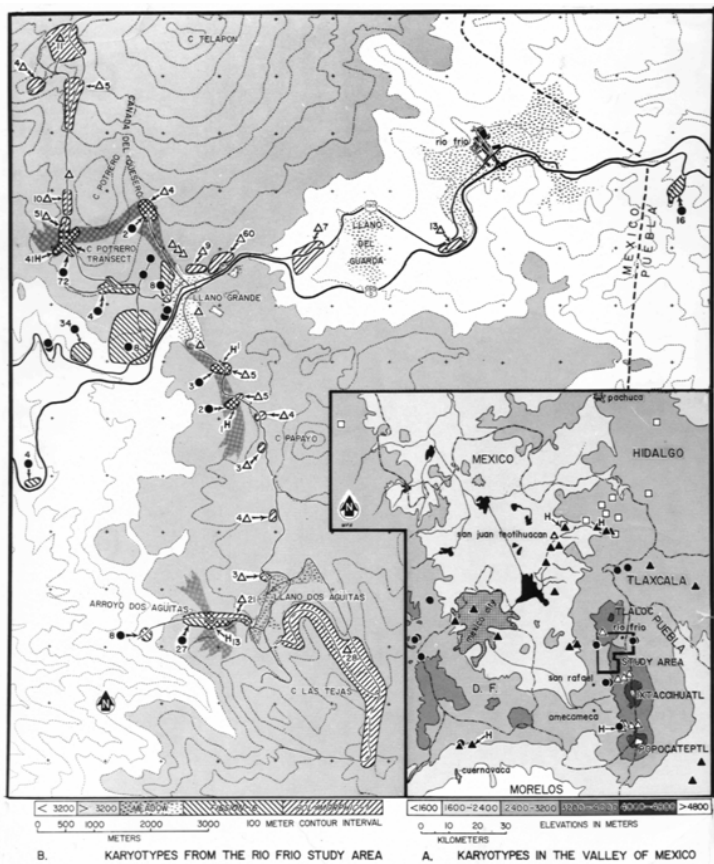


Fig. 11. A. Distribution of the karyotypically distinctive variants of *Sceloporus grammicus* in the Valley of Mexico. B. The F6 × P1 hybrid zone in the Rio Frio area E of Mexico City (from Hall 1973, Hall and Selander 1973). Locality symbols are defined in Fig. 10. See Marshall et al. (2006) for the most recent map of cytotypes in the Valley of Mexico area.

There may be one long sequence of chromosomal derivation from the standard karyotype: F5 → F5+6 → FM3 → FM1 → FM2, with F6 being independently derived from the standard/LS (or alternatively F5+6 is derived from F6, with F5 being independent). Standard/LS, F5, F6 and F5+6 all have wide, disjunct distributions: F5 along the western interior of the Mexican Plateau from near the US border in northern Chihuahua to the Valley of Mexico (Sites et al. 1996), and F6 along the lengths of the Sierra Volcánica Transversal from the Nevado de Colima in the west and the Sierra Madre Oriental to northern Nuevo León in the north. F5+6 is found at lower elevations along the east side of the Mexican Plateau from central Querétaro north to the lower Rio Grande Valley in Texas (Sites 1983). Wide geographic distributions and currently disjunct populations suggests that the F5<sup>[4]</sup>, F6, and F5+6 arrangements achieved their geographic limits no later than late stages of the Pleistocene or in the following Thermal Optimum (Hall and Selander 1973). The various FM races in this sequence of derivation are all found in northern parts of the Valley of Mexico that were blank on my map (Fig. 11a). The population I called P1 and Sites group calls HS exists in currently disjunct populations at high elevations on east and west sides of the Valley of Mexico and NW of Pachuca, generally above 3200 m (Sites and Davis 1989)<sup>[5]</sup>.

Sites and Davis (1989) compared isozyme, ribosomal and mitochondrial genetic relationships of six cytotypes.

Their observations are generally consistent with sequence suggested above but with some hints that this story may be too simple. Also the relationships of the F5 and FM3 cytotypes are unclear. F5 was not included in the Sites and Davis study, and FM3 was sampled from only a single site. For FM3, relationships based on nuclear vs mitochondrial are discordant.

Genomic analyses of the variation in Mendelian characters amongst seven of the eight chromosomal races meeting in the Valley of Mexico area (Sites and Davis 1989; Marshall et al. 2006) discriminated four “hypothetical evolutionary species” within the small area of the Valley of Mexico, but did not sample standard/LS, F6, or F5+6 from areas of their extensive distributions beyond the Valley. In these studies P1/HS and F6 seem to be the most distinct lineages and have been named as species: *S. anahuacas* (Lara-Góngora 1983) for P1/HS and *S. palaciosi* (Lara-Góngora, 1983) for F6. The other hypothetical evolutionary species recognized by Marshall et al. (2006) are standard/LS; and a complex comprised of F5+6, FM1, FM2 and FM3 that appeared to form a single evolutionary species by their genomic criteria. Marshall et al. did not include F5 in their study. Sites (1982, 1983) found no evidence for specific morphological distinctions among the three races: standard/LS, F6 and F5+6 along the northern half of the Sierra Madre Oriental. However, given the still limited geographic sampling of genomic variation, I believe that is premature to apply formal species or subspecies names to any of these populations until comparable chromosomal and genomic studies have been completed of all the chromosomal races over the extent of their ranges.

Parapatric contacts have now been mapped for 6 different combinations of chromosomal races in central Mexico. (Hall and Selander 1973; Hall 1973; Arévalo et al., 1991, 1993, 1994). Hybridization (based on heterozygosity for chromosome markers that are fixed in populations outside the contact zone) is found between the contacting populations in all of these contacts. Where hybrid zones have been mapped in detail, they are narrow. In three places where Hall and Selander (1973; Hall 1973 - Fig. 11b) located the contact between HS and F6, the hybrid zone was on the order of 500 m across, not much greater than the potential dispersal distance of a single individual. Putative F<sub>1</sub> hybrids were frequent in the middle of the hybrid zone with deficiencies in backcross or F<sub>2</sub> genotypes. Arévalo et al (1993) located narrow hybrid zones between HS and F6 and F6 and LS above Ajusco on the west side of the Valley of Mexico, but were unable locate individuals in mid-contact areas or collect large enough samples for detailed analysis. Arévalo et al. (1993), Sites et al. (1993, 1995, 1996), and Marshall and Sites (2001) found the hybrid zone between F5 and FM2 near Tulancingo to be about 1 km wide, with strong deficits in heterozygotes and linkage disequilibria. Based on mark and recapture and genetic studies qualified by many caveats, they estimated average dispersal

distances for juveniles and females to be less than 100 meters with dispersals for males possibly to be somewhat more. Except for the various combinations of contacts between multiple fission populations in the Valley of Mexico north of San Juan Teotihuacán, there is no reason to believe that any of the other contacts happened recently (i.e., post Pleistocene).

Despite evidence for reduced fitness in chromosomally heterozygous individuals in the contact zones and close association between chromosomal heterozygosity and heterozygosity for Mendelian genes, in male meiosis, there is little evidence chromosomal nondisjunction in male fission heterozygotes contributes to reduced fitness (Reed et al. 1995, 1995a). However, female heterozygotes in the F5 × FM2 contact near Tulancingo show reduced fecundity and higher fetal inviability (Reed and Sites 1995; Sites et al. 1995). Although female meiosis remains to be studied, Reed and Sites attribute the reduced fitness to genic difficulties rather than chromosomal nondisjunction.

## Discussion

In my thesis (Hall 1973, 1977) I attempted some theoretical answers to the question that first led me to study phrynosomatine lizards, “*Why and how has Sceloporus evolved so many more species than have other genera of related and older origins, and might understanding these questions provide a more general insight into problems of species formation and adaptive radiation?*” This section reviews the theory and how it stands up against the large amount of subsequent phrynosomatine data summarized above.

I note that there are other radiations (e.g., *Anolis* – Poe 2004, *Liolaemus* – Torres-Pérez et al. 2009, morabine grasshoppers – Kawakami et al. 2009, Mus around the Mediterranean – Piálek et al. 2005, and mole rats – Nevo et al. 2001, etc.) showing similar concatenations of chromosomal variability, often with narrow hybrid zones paradoxically associated with chromosomal and genic differentiation, apparently rapid formation of evolutionary species, limited vagility, and subdivided populations that are beyond the scope of the present work to review. However, the phrynosomatine radiation is now well enough known to illustrate and provide some preliminary tests of the theory, and I leave it to those who are more familiar with other relevant groups to test the applicability of the ideas presented here to their preferred radiations.

### *Species and speciation in the grammicus complex*

In a local ecological framework, an evolutionary species is a lineage that evolves independently of genic contact with adjacent populations (Marshall et al. 2006; Baker and Bradley 2006). Morphological variation may be a poor indicator of barriers to gene flow or sequences of derivation (Sites 1982, 1983; Sites and Marshall 2003, 2004; Smith 2001).

Around the circumference of the Valley of Mexico, the grammicus cytotypes P1/HS (*S. anhuacus*) and F6 (*S. palaciosi*) are enough different morphologically from standard/LS and each other that Lara-Góngora (1983) recognized them as species. However, Lara-Góngora’s study of morphological variation was limited to the Valley of Mexico and the surrounding mountains. Sites and Dixon (1981) and Sites (1983) showed that for standard/LS, F6 and F5+6 races over their northern ranges beyond the Valley of Mexico, there seems little concordance between morphology (presumably adaptive to local ecologies) and diagnostic chromosomal differences (which may trace back to bifurcations tens to hundreds of thousands of years or more).

On the other hand, where chromosomally different *grammicus* meet geographically, genic and chromosomal markers highlight barriers to gene flow over distances of a few hundred meters to a kilometer. Apparently effective barriers to exchanging nuclear genes exist in all the studied contacts between different chromosomal races (P1/HS × F6 – Hall and Selander 1973, Arévalo et al. 1993), standard/LS × FM2 – Hall (1973), standard/LS × F6 – Arévalo et al. 1993, and F5 × FM2 – Marshall and Sites 1993). At least adjacent to their contact zones, given the extents of genic differences, the first three pairs are evolutionary species whose parent populations may have been evolving independently since the Pleistocene. On the other hand, the FM2 lineage may be a more recent derivative of a sequence of derivation starting with F5 (or F6).

Assuming that the four chromosomal differences diagnosing the F5 and FM2 races were sequentially fixed, rather than via some mechanism of assortment in a population that was originally polymorphic for all of the fissions, the contact between F5 and FM2 would be secondary; between an ancestral lineage with a disjunct range (F5) and a recent and highly derived one (FM2). To help understand the formation of the FM races as evolutionary species (if that is what they are), the most interesting contacts would be possibly primary ones between F5+6 × FM3, FM3 × FM1 and FM1 × FM2 – possibly representing their derivation sequence (Sites and Davis 1989). The known ranges of the three FM races in the northern Valley of Mexico are all restricted to a single small area approximately 160 × 100 km (as estimated from fig. 2 in Sites and Davis 1989), see also Fig. 11a. Although punctuated by volcanic mountains and cones, this is an area of traditional dry-land agriculture where *grammicus* commonly take cover in cultivated *Agave* and *Opuntia*, and stone walls surrounding fields (Hall 1973; Sites et al. 1995). The sequence of chromosomal derivation in this area appears to begin with (1) F5+6 – the presumptive ancestor on the north or west, followed by (2) FM3 in the north and east, the (3) FM2 in the south and east, and most recently by (4) FM1 in the north and west of the area. As shown by the Sites group’s maps, F5+6 and three FM races are interdigitated among and may have

displaced earlier populations of standard/LS, F5, F6 and P1/HS. It is plausible that fixations of marker chromosomes diagnostic for the FM1, FM2, and FM3 races may have been contemporaneous with the spread of traditional native American cultivation of *Agave* and *Opuntia*. This style of agriculture probably began more than 2500 years ago with the growth of large populations in the Valley of Mexico (Millon 1970; Parsons 1974). Thus the contacts between F5+6, FM1, FM2 and FM3 would offer the most recent primary contacts between compact populations that might still be considered to be unfragmented evolutionary species. Unfortunately, none of these contacts have been studied.

#### *Hybrid zones as sinks for gene flow*

Based on evidence from the *grammicus* hybrid zones he studied, Hall (1973, 1977) presented the idea that contact zones involving hybridization between populations with limited vagility would act as “sinks” for gene flow if hybrids and backcrosses showed reduced fitness. Reduced fitness of hybrids and recombinants would decrease population pressure within the zone compared to “pure” populations on either side of the zone. This would attract net dispersal of individuals from near the edges of the zone towards the centre where the competition for limiting resources is reduced (e.g., as described in the F5 × FM2 hybrid zone by Marshall and Sites 2001). Irrespective of linkage, many genes carried towards the center of the zone by net dispersal will be lost in less fit hybrid and recombinant genotypes.

Key (1968, 1981), White et al. (1969), and White (1978a) called hybrid zones resulting from reduced hybrid fitness between populations of limited vagility, “tension zones”. Such tension zones were expected to be geographically located where fitnesses of the parent forms were approximately equal. They might also be “pinned” to locations in the environment where there were impediments to dispersal (such as marginal habitats or narrow connections – e.g., the Street of the Dead for the standard/LS × FM2 contact in the Teotihuacán archeological zone. Key (1968) also suggested that hybridization in a tension zone would tend to minimize sharp curves along the length of the contact because the pure population on the concave side of the contact would have a longer front than would the pure population on the convex side, and thus would be able to send more dispersing individuals towards the zone than the convex population could. However, neither Key nor White emphasized that these properties were literally due to reduced population pressure from less fit hybrids in the center of the zone.

Somewhat contrary to Key’s (1968) idea that elastic “tension” along the hybrid zone would tend to straighten out contacts, both the P1/HS × F6 contact near Rio Frio (Fig. 11b) and between F5 and FM2 near Ajusco (see maps in Sites et al. 1995; Marshall and Sites 2001) show sharp bends. In both cases ecological gradients in the landscape are linear. The respective pure populations are

associated with particular environmental features (e.g., oak trees vs stone walls, *Yucca* and *Agave*), and the long axis of the hybrid zone tends to follow the contacts of these features. This suggests that the respective “pure” populations may have significantly different genetic adaptations in accord with comparatively long evolutionary separations.

Mitochondrial DNA, inherited clonally through females, may have the capacity to introgress beyond the limits of the hybrid sink as mapped by chromosomal or nuclear gene differences (Marshall and Sites 2001; see also McGuire et al., 2007; McKay and Zink 2009). In most vertebrates, females disperse less from their birth locations than males, and thus, depending on sex differences in average dispersal distances, introgressed mitochondrial gene differences may be partially protected from being drawn back into the sink.

In sum, Marshall and Sites (2001) provide fairly clear observational evidence for a sink effect in the Tulancingo contact between F5 and FM2 *grammicus* populations.

#### *Chromosomal variability and cascading speciation*

Given that the more recent reconstructions of subclades within the Phrynosomatinae have relied mainly on genic evidence rather than morphological or chromosomal evidence, the phrynosomatines provide an excellent framework where comparative approaches can be used to explore possible roles chromosomal variation in *Sceloporus* may have played in the radiation of its species by comparison to the chromosomal conservatism of its sister phrynosomatine genera.

(Hall 1973, 1977) proposed a theory of cascading speciation involving genetic revolutions (Mayr 1954, Carson and Templeton 1984) that might account for the sudden proliferation of species showing particular styles of chromosome mutation. In abbreviated form, the initial theory made three assumptions (see Hall 1977 for a complete exposition and comparison with related theories):

1. *Negatively heterotic mutations.* Negatively heterotic chromosomal mutations can plausibly be fixed by chance in any small deme that is inbred enough to have an effective size of 10 or less at the time of mutation. Fixation can occur without complete isolation if immigration is less than 10-20%. Although individual mutations are rarely fixed, over evolutionary time in species with subdivided populations, many such mutations may achieve local fixation in demes somewhere over the species’ range for at least for a few generations at a time.

2. *Hybrid sink formation.* If through chance or local selective effects, the chromosomally derived population expands enough to protect its central demes from hybridization, a hybrid sink as described above can form to allow further genic differentiation.

3. *Genetic systems and gene sampling by founder populations.* The detailed functioning of a lineage’s genetic system largely determines (a) the probability of

fixation of chromosome mutations in local populations and (b) population's ability to form a sink after fixation. Many genes affecting the probability of fixation of negatively heterotic chromosome mutations in demes may have nearly neutral fitnesses in chromosomally homozygous individuals, because they reduce fertility only in the rare heterozygous individual. Yet, other aspects being identical, demes having the highest frequencies by drift of alleles favoring chromosomal speciation would most probably found new species. A species derived from such a founder population would tend to perpetuate a higher frequency of favorable alleles than would the parent species, and thus would be more likely to found further new species by the same kind of mechanism. Although there are many cases where chromosomal heterozygosity does not significantly affect fertility, in some organisms heterozygosity for chromosomal rearrangements is known to cause some level of malassortment in meiosis (Ford and Evans, [1973](#); Searle [1993](#); Winking et al. [2000](#); Scascitelli et al. [2003](#), [2004](#), [2006](#)).

Assuming a chromosomal mutation is negatively heterotic, heritable factors of population biology may profoundly affect the probability that it can achieve local fixation. For example, individuals in many *Sceloporus* populations cluster around dispersed refugia that provide escape cover for only a small number of individuals (e.g., *grammicus* are closely associated with widely scattered rotting logs), where effective population size may be further reduced by male dominance (one dominant male may inseminate most females using the refugium) and the fact that if a new mutation occurs early in the germ line, all of the progeny of that first mutant individual may carry it.

Working from these prior assumptions and a general understanding of genetic systems, an explanatory theory emerges:

*Speciation can be a cascading process.* Genetically controlled aspects of each species' genetic system vary over the species' geographic range (e.g., Thompson and Woodruff [1978](#), Zhao et al. [2004](#), Baer et al. [2007](#)). Nascent speciation will most frequently occur in areas of the parent species' range where heritable aspects of the genetic system are more favorable. In its initial differentiation, a population founding a nascent species will sample this relatively favorable genetic background. Thus, properties of the derived species' genetic system will be more favorable to further speciation than the average properties of the parent species' genetic system. This non-random sampling may serve to amplify the frequency of genes (i.e., "genetic amplification") favorable to further speciation in the derived species, and so on for further speciation events. This may lead to a burst of rapid speciation that I called a cascade (Hall, [1973](#)).

Examples of the kinds of genes affecting the probability of chromosomal speciation might be ones whose alleles influence the frequency of Robertsonian mutation (Ives, [1950](#); Zhao [2004](#); Kalitsis et al. [2006](#)) or

malassortment from Robertsonian trivalents in meiosis. These would have minimal effects on individual fitness of chromosomally normal individuals, but might strongly affect the probability of speciation. Other genes, such as those controlling vagility, male dominance, chiasma frequency and localization etc. would affect the degree to which locally adaptive genes accumulate and assort with derived centromeres, and the degree to which a sink effect could work to protect those locally adaptive genes from swamping by introgression from ancestral populations.

*Chain growth.* Assume that a chromosomally derived species inherits the bulk of its genes from the sample included in the original chromosomally differentiated deme. Given the minimal effects on individual fitness of alleles "favorable" for speciation, the chromosomally derived species should perpetuate for long periods increased frequencies of these alleles. Because of this selective founder effect, the average frequency of such favorable alleles would be higher (possibly much higher) in the derived species than in the ancestral species. Therefore, the first chromosomally derived species ("S<sub>1</sub>") would be more likely to initiate further derivations, ("S<sub>2</sub>"), than would be the ancestral ("S<sub>0</sub>") species. (Subscripts denote the order in which species form in a sequence of derivation.) And, of course, the same kind of amplification process might be repeated when the S<sub>2</sub> species was formed so that this S<sub>2</sub> species would be more likely to form an S<sub>3</sub> species than either the S<sub>0</sub> or S<sub>1</sub> would be to form another species. This process might continue to concentrate or amplify the frequency of favorable genes for several generations of increasingly rapid speciation to form a cascade of species.

*Chain termination.* Assuming that a cascading sequence of speciation begins, three processes may work individually or collectively to halt proliferation.

(1) *Termination by substrate exhaustion.* As in *Sceloporus*, some apparent sequences of derivation are characterized by particular styles of chromosome mutation (e.g., centric fissions *or* fusions) that may be favored by particular aspects of the genetic system. Such chains would be terminated when the available chromosomal substrate for that kind of mutation was used up, to result in terminal species either with all acrocentric or with all metacentric chromosomes.

(2) *Termination by counter selection.* The cascading process may fix genes favoring speciation whose negative effects on individual fitness in turn select for other components of the genetic system to counteract the effects of the fixed "favorable" genes. For example, if cascading amplification increases rates of spontaneous fissioning, the situation may arise where chromosomal heterozygosity becomes frequent. Here, selection would favor evolution of the meiotic spindle apparatus in directions that insured balanced assortment from chromosomal heterozygotes. In the case of Robertsonian mutations, this is easily achieved by insuring: (a) that chiasma formation reliably links the metacentric and the

two acrocentric arms into a trivalent, and (b) that the centromeres of the trivalent always orient alternately (see diagrams in Hamerton, 1971).

(3) *Termination by niche saturation*. If nothing else halted a cascade, available and accessible ecological niches which could be occupied by a radiation would eventually become sufficiently saturated to prevent further successful speciation.

#### *Expected phylogenies from cascading speciation*

Hall (1977) explains patterns of chromosomal variation that might be predicted to occur from cascading speciation.

1. Sequences of chromosomal derivation should be mainly linear from origin to termination.
2. The sequence of ecological or geographic derivation will closely parallel the sequence of karyotypic derivation.
3. Species ending a sequence will either be a) ecologically very specialized or b) ecologically dominant compared to near relatives.
4. Side branches in a sequence will usually either be close to the beginning or end of the sequence.
5. Missing species in a sequence will usually be chromosomally intermediate.
6. Many terminal species will have "used up" their chromosomal substrate for speciation.
7. Species polymorphic for the kind of chromosomal mutation involved in a sequence of derivation will usually be terminal.

#### *Testing the theory against the phrynosomatine radiation*

Chromosome diversification in *Sceloporus* should be considered in relation to the phyletic and ecological diversification of the phrynosomatine lineages shown in Fig. 6 (after Presch 1969). Excepting that *Petrosaurus* is now considered to be a sister to *Uta*, *Urosaurus* and *Sceloporus* - including *Sator*, recent phyletic studies accord with Presch's view in Fig. 6 (Wiens 1993; Reeder 1995; Reeder and Wiens 1996; Flores-Villela et al. 2000; Schulte et al. 2003). As discussed by Hall (1973) most *Sceloporus* species appear to be predator limited rather than food limited, and thus are under selective pressure to evolve escape strategies. Common strategies are: being very cryptic, shimmy burrowing in loose sand and soil, running to burrows in the ground or other holes, hiding in grass clumps, moving out of visual range (e.g., moving to the other side of a rock or tree trunk), and using crevices in dry woody material (under bark, splits in decaying logs, etc.) or in rock outcrops (e.g., under exfoliating granite). Most character displacement allowing sympatry to develop seems to have been related to structural dimensions of the environment (Hall 1973). The evolution of viviparity has also enabled some *Sceloporus* to exploit areas at high elevation and/or high humidity where growing seasons are short and soil may be too cold or too saturated for egg incubation (Guillette et al. 1980;

Benabib et al. 1997; Méndez de la Cruz et al. 1998; Andrews 2000).

The patterns of chromosomal phylogeny provided by the five sequences of chromosomal derivation seen in *Sceloporus* show some common features that may result from basic properties of a cascading speciation process:

1. All sequences of derivation except the 2n=40 *clarki-melanorhinus* complex end with populations which have either (to a first approximation) completely fused or completely fissioned (at least as polymorphisms) karyotypes (See Fig. 7, Fig. 8, Fig. 9). Satisfies prediction 1.

2. In all sequences except for the very recent *grammicus* case, there are few or no chromosomally intermediate survivors, and in *grammicus*, towards the end of the fissioning sequence, chromosomal intermediates and the terminal populations have small geographic ranges within bounds of the range occupied by the standard/LS race (Sites and Davis 1989). Satisfies prediction 2.

3. In the two sequences where there definitely are intermediate survivors (within *grammicus* and the sequence to the 2n=22 *horridus* - including *undulatus* and *formosus* groups), the karyotypes of these survivors suggest that long sequences of derivation have nearly linear phylogenies. The only apparent exceptions to this linearity might be: (a) the P1/HS *grammicus* population, which may be derived from F6 by a re-fusion of the fissioned pair 6, rather than from the karyotypically more similar standard/LS populations; (b) presumably one of F5 or F6 gave rise to the F5+6 race, leaving the other as a side-branch; and (c) the 2n=26 mainland *magister*, which is derived away from its presumed place in the linear sequence by a pericentric inversion. Mostly satisfies predictions 3, 4, 5 and 6.

4. Beyond single localities, the only known cases of Robertsonian polymorphism in *Sceloporus* involve the terminal and near terminal FM populations of *grammicus*, or the P1/HS *grammicus*. Significantly, all polymorphisms in *grammicus* involve only the larger and most exactly metacentric chromosomes. These chromosomes would be expected to show the most regular (i.e., balanced) orientation on the meiotic spindle. Satisfies prediction 7.

5. Lastly, but most interestingly, in all sequences of derivation except for the very recently terminated *grammicus* (which may have reached its present state only with the development of native American agriculture in central Mexico), the chromosomally terminal forms are widespread and ecologically important radiations: (a) the 2n=46 *merriami* has seven subspecies and is common in disjunct areas of major rock outcropping from the Big Bend region of the Rio Grande Valley south to the barrier of the sandy deserts of south-central Coahuila and northern Durango (Smith et al. 2003). (b) The 2n=40 *clarki-melanorhinus* complex occupies tree and rock habitats from Guatemala north into the SW US along the Mexican Pacific and Gulf of California coasts and slopes (Smith,

1939; Smith and Taylor, 1950). (c) The  $2n=24$  *scalaris-aeneus* complex occupies grassy habitats over most of the Mexican Plateau from Oaxaca north to southeastern Arizona and adjacent New Mexico (Mink and Sites 1996; Creer et al. 1997). (d) The  $2n=22$  complex of mostly parapatric large-scaled species is the dominant or near dominant trunk-ground-rock lizard in most habitats of North America from western Panama to the northern limit of lizard distribution. These  $2n=22$  lizards are missing only from the majority of Baja California, the most extreme deserts, the southern part of the lower Mississippi Valley, and the lowlands of Veracruz. Satisfies prediction 3.

#### Chromosomal variation and rates of evolution

Chromosomally conservative phrynosomatine species also appear to be evolutionarily conservative. The geographic distribution of the chromosomally primitive species of *Sceloporus* is suggestive of relict populations that radiated outward from origins associated with the formation and growth of the North American deserts (Axelrod 1979). Although a detailed phylogeographic review of speciation patterns in *Sceloporus*' sister clades forming the Phrynosomatinae is beyond the scope of the present work, the wealth of phylogenetic data available for the radiations of *Phrynosoma* (Upton and Murphy 1997; Zamudio et al. 1997; Leaché and McGuire 2006); the sand lizards, *Uma*, *Holbrookia* and *Callisaurus* (Wilgenbusch and de Queiroz 2000; Lindell et al. 2008); *Sceloporus*' sister clades *Petrosaurus* (Aguilars-S et al. 1988), *Uta* (Upton and Murphy 1997; Hollingsworth 1999), and *Urosaurus* (Lindell et al. 2005) all seem to tell the same story. All these genera seem to be centered in the warm North American deserts, where much of their speciation is consistent with geographic fragmentation of lineages already existing in the Miocene and Pliocene, contemporaneous with origins and evolution of the North American deserts and the opening of the Gulf of California to form insular land masses, eventually coalescing as the present day Baja California Peninsula (Hall, 1973; Sites et al. 1992; Murphy and Aguirre-Léon 2002; Riddle and Hafner 2006; Riddle et al. 2008).

Chromosomally primitive generalist *Sceloporus* are found around the edges of the hot deserts with apparent vicariants found in sub-humid, semi-arid and thornscrub habitats, as illustrated by the distribution of *S. variabilis* (Sites and Dixon 1982) and its relatives on the eastern slopes of Mexico; the southwestern distributions of *siniferus*, *utiformis*; and their relatives (*Sator*) *angustus* and *grandaevus* isolated on islands in the southern Gulf of California. Phylogenetic analyses (e.g., Leaché 2009) suggest that the bifurcation between the eastern “variabilis” and the southwestern “siniferus” is one of the oldest in *Sceloporus*. The highly specialized bunch-grass users,  $2n=46$  *merriami*, then seem to be the next oldest branch (e.g., Leaché 2009). Following this there is an apparent bifurcation between the southwestern generalists and the *jalapae* group now found in

relatively dry areas of the plateau. The basal  $2n=34$  stock apparently ancestral to the major radiation of chromosomally derived *Sceloporus* (i.e., *pyrocephalus*, *nelsoni*, *licki*, *hunsakeri* and *orcutti* - Fig. 8) all seem to be vicariants associated with the rifting of the Baja California Peninsula from the mainland and the opening of the Gulf of California (Hall 1973, Hall and Smith 1979; Sites et al 1992).

Large-scaled species deviating from the pleisiomorphic  $2n=34$  pattern appear to have evolved more rapidly and have substantially diverged ecologically and geographically from their chromosomally conservative relatives listed above. Chromosomal derivation seems to have begun with a  $2n=32$  form that no longer exists (Fig. 8), but that would have been basal to the  $2n=32$ ♀,  $31$ ♂ *asper* and the wood and rock crevice users, plus the  $2n=40$  *clarkii* group members; respectively radiating out from west to east over the Mexican Plateau and southward in thornscrub and tropical deciduous forest along the western and southern coast of Mexico. Two  $2n=30$  complexes exist in the west (Fig. 9: (*zosteromus/rufidorsum*) in the Baja California Peninsula and *graciosus* extending from pine and oak forests of the northern highlands of Baja California through the dry montane areas of the western United States into southern Canada. In Baja California there is some overlap between the  $2n=30$  species and the  $2n=34$  ones – with  $2n=34$  *orcutti* being dominant on the Gulf slopes of Baja California Norte and  $2n=30$  *zosteromus* dominant on the Pacific slopes. Sites et al. (1992) note that karyology of the *zosteromus* complex is still poorly understood – especially given that no representatives from the Gulf of California islands have been karyotyped. No forms with a  $2n=28$  karyotype are known. The  $2n=26$  *magister* complex radiates out from the northern Baja California Peninsula and Sonoran Desert around the head of the Gulf of California with little genetic evidence for speciation within the complex (Grismer and McGuire 1996; Leaché and Mulcahy 2007). Leaché and Mulcahy estimate the divergence of the  $2n=30$  *zosteromus* and  $2n=26$  *magister* complex took place around 5.5-6.5 Ma with diversification within *magister* itself beginning 3.4 Ma. Based on genic evidence (Leaché 2009; Flores-Villela et al 2000; Wiens and Reeder 1997) the  $2n=24$  *scalaris* group is sister to the  $2n=22$  radiation. This is not contradicted by its geographic distribution, where its inconspicuous and predominantly ground-dwelling species are found across most of the Mexican Plateau except for the Chihuahuan Desert (Mink and Sites 1996).

Based on morphological and genomic data (Leaché 2009; Flores-Villela et al 2000; Wiens and Reeder 1997; Smith 2001) the  $2n=22$  species, comprising the *horridus/spinosus/undulatus* complex of species together with the *formosus* species group appear to be the most recently derived. This complex includes some 20-25 species. Given the genomic evidence, there is little doubt that the  $2n=22$  species form a natural clade. Excepting substantial overlaps between oviparous

species and the viviparous *formosus* species, the 2n=22 species form a mostly mosaic distribution over essentially the entire range of *Sceloporus* outside Baja California and the deserts around the head of the Gulf of California. Interestingly, there appears to be little correlation in *undulatus* between phylogenetic relationships and the morphologically recognized subspecies (Leaché and Reeder 2002; Miles et al. 2002). The 2n=22 *S. virgatus* is an early branch in the *undulatus* radiation (Leache and Reeder 2002). Mitochondrial genomic variation within its mountain isolates (Tennessen and Zamudio 2008) suggests that *virgatus* populations on different mountain ranges have been genetically isolated for at least hundreds of thousands if not more than a million years – despite evidence that suitable habitat corridors for migration between the populations existed as recently as 24,000 years ago. Smith's (2001) study of mitochondrial phylogenetics in the *formosus* group, estimates its divergence from *horridus/spinosus* complex around 5.4-7.2 ma, with the divergence between *horridus* and *spinosus* between 3.1-3.4 ma, and with geographic expansion and divergences within *formosus* beginning soon after the separation from *spinosus*. The overlap of time spans for divergences of species in the sequence from 2n=30 to 2n=26, and early dichotomies between the 2n=22 species suggests this process may have occurred very rapidly on an evolutionary timescale.

The inconsistencies between the mitochondrial and nuclear genetic phylogenies among the chromosomally derived species reported by different authors, (e.g., Wiens and Reeder 1997, Flores-Villela et al 2000, Smith 2001, Leaché and Mulcahy 2007, Leaché 2009; Wiens et al. 2009) suggest the chromosomal differences in *Sceloporus* (other than the recently derived *grammicus*) were established during a period of rapid speciation where there was insufficient time for clear lineage sorting to occur. Some of the inconsistencies involving nuclear genes may also reflect the use of single individuals to represent a species or population.

The sequence of derivation beginning with a 2n=30 and ending in the 2n=22 species is a picture of accelerating evolution and proliferations of species – perhaps within the late Miocene or Pleistocene (Leache and Sites [in press](#)). It cannot be determined how much of this picture is an artifact of the fact we do not see extinct species exterminated in climate changes or competitively replaced by newer ones; or that it is a genuine depiction of the facts. However, it seems there was enough ecological differentiation in association with karyotypic changes that newer clades were able to expand their ranges to involve at least some sympatry with older clades, such that subsequent geographic speciation within the expanded clades was also possible. And, certainly, what is by all accounts the most recently derived 2n=22 clade has proven to be exceptionally plastic in its ability to overlap most other *Sceloporus* outside the extreme deserts.

The main exceptions to linear sequences of derivation are provided by the large proliferations of the closely related *torquatus* and *grammicus* groups – assumed to be an early branch of the sequence of derivation leading to the 2n=22 species groups. The several species of the *torquatus* group (Smith 1939; Hall 1973; Martínez-Méndez and Cruz 2007) are all viviparous and specialized to use crevices in rock outcrops at middle and middle to high elevations for escape cover (excepting *cyanogenys* found at lower elevations, and *serrifer* in Chiapas, Guatemala and Yucatan using trees at low elevations), while the viviparous *grammicus* (Hall 1973) use cover in cracks and holes in woody objects. The *torquatus* group range extends from southern Arizona, New Mexico and Texas south to Chiapas, Yucatan and northern Guatemala, with some areas of sympatry between different sized species. The *grammicus* group extends over the entire Mexican Plateau from the northern limits of the Mexican plateau and southern Texas south to Oaxaca.

There is no suggestion that chromosomal variation was involved in the early proliferation of crevice users (Hall 1973). Allopatric speciation within the *torquatus* group rock crevice users can be easily accounted for by their restriction to persistent islands of rock outcrops that that remain isolated over geological time scales. Similarly, early speciation in the *grammicus* species group is easily explained by Pleistocene climatic fluctuations and barriers along the Sierra Madre Occidental (Hall 1973). However, the remarkable chromosomal variation within the *grammicus* complex suggests a comparatively recent or even on-going series of speciation events involving Robertsonian fissions.

#### *Association of chromosome mutation and speciation*

The comparative data reviewed above show a strong correlation between chromosomal variability and apparently rapid speciation – at least in the lineage of large-sized, large-scaled *Sceloporus*. Aside from the chromosomally variable *grammicus*, the comparative evidence also suggests that the chromosomally derived lineages of *Sceloporus* are either ecologically dominant and able to establish relatively continuous populations over wide geographic areas by dominating good perching sites associated with open ground and a variety of cover, or are very specialized (e.g., as *merriami*).

Sites and Moritz (1987) proposed three classes of speciation models involving chromosomal change: (I) fixation in small demes of individual mutations reducing heterozygous fitness due to unbalanced assortment, (II) independent fixation of fusions/fissions for different chromosomal arms in different demes such that heterozygotes formed in secondary contacts between the different demes form “monobrachial” trivalents or multivalents leading to a high probability of unbalanced assortment, and (III) rearrangements producing “recombinational break-down” leading to the disruption in internally balanced linkage groups by recombination (attributed to Shaw 1981 and Shaw and Coates 1983).

The linear sequences of chromosomal differentiation seen in *Sceloporus* are inconsistent with class II speciation, but do not refute Class I models of cascading chromosomal speciation or that chromosome differences may facilitate the establishment of hybrid sinks as barriers to gene flow. However, none of the *Sceloporus* studies reviewed unambiguously support a model of chromosomal speciation where fixation of the Robertsonian mutation of and by itself is sufficient to precipitate speciation. Nor do they completely refute such a model.

White (1978a) argued that the close association between chromosomal rearrangements and speciation in animals of limited vagility strongly supported the idea that rearrangements were directly involved in the “stasipatric” speciation process, but not necessarily on a 1 mutation : 1 species basis (White 1978b). White (1978b) and Riesberg (2001) both propose that the rearrangements may be adaptive by protecting locally coadapted genetic combinations on the rearranged chromosomes from being disrupted by introgression from neighboring populations with different adaptations. This is basically the same argument as Sites and Moritz’s (1987) Class III speciation, but from the point of view of the core population. White proposed that local populations of all but the most vagile species exhibit “area effects”, where some geographic populations are characterized by allele combinations that are rare or absent elsewhere. Any chromosome mutation that served to protect local adaptations from being disrupted by immigrants should have a reasonable chance of establishment and fixation. For this mechanism to function, the chromosome mutation must spread until it coincides with the area effect.

In *Sceloporus* it is possible that Robertsonian mutations affect recombination in chromosomal heterozygotes. Unpublished observations from my original score cards<sup>[2]</sup> of male diakinesis indicate that chromosomally conservative iguanids, as well as the relatively conservative crevice users are characterized by two or more chiasmata along each arm of the larger macrochromosomes, while highly derived species show one or two strictly terminal chiasmata per bivalent in fissioned lineages such as *clarkii/melanorhinus* or FM *grammicus* (Fig. 3) or a single centromerically localized chiasma as in some 2n=22 species (Fig. 5). Interestingly, in diakinesis arrays from fission heterozygotes illustrated by Porter and Sites (1985), the chiasmata of the fission trivalents are terminal or nearly terminal, while chiasmata of the bivalents seem to be more randomly located along the length of the chromosomes. Such mechanisms of chiasma localization could protect long stretches of each chromosome arm from recombination, allowing the accumulation of locally coadapted gene complexes.

## Conclusions and unanswered questions

Based on the work reviewed here *Sceloporus* offers a rich natural laboratory for exploring the most difficult questions of what species are and how they form and evolve through time. The fact that many of these questions are still open and interesting after the publication of more than 100 research papers over some 40 years testifies to the difficulties of inferring population level processes working over many generations from our limited samples of a few individuals from a few points of time and geography.

To this point, the major conclusions from these decades of work are:

1. Combined genomic and chromosomal studies have provided a robust and detailed phylogeny for all of the phrynosomatine lizards able to support comparative studies on many aspects of their biology.
2. There is strong but not conclusive evidence that chromosomal differentiation is directly associated with the formation of some species in *Sceloporus*.
3. There is suggestive evidence that chromosomally variable lineages in *Sceloporus* have evolved more rapidly (whatever that means) and have formed more species than chromosomally conservative lineages.
4. Chromosomal differences have facilitated the identification and mapping of narrow hybrid zones that appear to act as effective barriers or “sinks” for gene flow between the hybridizing populations. Although not proven, these chromosomal differences may be directly involved in the dynamics of the barriers to gene flow.

The studies also highlight some specific questions for further research whose answers may illuminate roles chromosomal mutations may play in speciation and hybrid zone dynamics.

1. Karyotypes from *Sceloporus* species around the Gulf of California are based on single individuals or small sample sizes and do not represent the insular populations from the Gulf of California. The known range of karyotypic variation between and within the *magister*, *zosteromus/rufidorsum*, and *clarkii/melanorhinus* complexes would justify more detailed geographic sampling of their karyotypes.
2. Gene sampling for phylogenetic studies should be extended to detect intrapopulation polymorphisms and geographic variation.
3. Different species of *Sceloporus* differ greatly in terms of chiasma frequency and localization in male meiosis. What are the details of this variation between species and between different populations within species? Does it co-vary with different degrees of chromosomal derivation. To what extent does this vary with the reproductive cycle and animal condition?
4. Although *S. grammicus* would not be as tractable to work with in the lab as *Mus*, *grammicus* born in the spring are capable of breeding in their first autumn

- (Guillette and Casas-Andre 1980), which suggests that it would be practical to study meiotic pairing and assortment in progeny from controlled matings of chromosomally different parents.
5. Many aspects of the fixation of chromosomal mutations would benefit from detailed computer modeling of demic structure, and hybrid zone dynamics. These should be associated with field studies focused directly on comparative studies of the dispersal behavior and population structure of species from chromosomally variable vs chromosomally conservative lineages.
  6. The development of non-lethal and minimally invasive methods for the precise mapping of genomic and chromosomal variability that allowed tracking over several years would be greatly welcomed. From my own experience, the methods I used for population sampling caused great disturbance to the existing populations and the physical habitat, such

that follow-up studies would be meaningless for several lizard generations.

Finally, to build on the value of the preceding studies - if it is not already too late, I would urge that minimally invasive field studies be given high priority while *Sceloporus* habitats remain that have not been disrupted by the agricultural and space requirements of ever-increasing human populations.

Smith's (1939) statement about *Sceloporus* that "Attractive problems in species formation and geographical distribution are presented, and their solution is brought within reach by the fact that these lizards are frequently abundant and relatively easy to observe and collect", is more true today than it was 70 years ago when he completed his foundation monograph because we already know a great deal about the group to help us formulate deeper and more precise questions.

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The long hiatus following my last publication on *Sceloporus* in 1983 and my failure in the 1980s to respond to peers in evolutionary biology deserves some explanation. After years of marginal teaching positions with no facilities to continue research following completion of my PhD in 1973, in 1980/81 I failed to find any employment that offered a prospect that I might ever resume research. Poverty left no alternative but to build a new career working in industry with information and knowledge management systems. Severe depression from having to leave a discipline I loved made it impossible to deal with the many loose ends of my research career.

## Endnotes

- [1] Since my thesis work was completed (Hall 1973, [1977]) the constitutions and nomenclatures of family, subfamily and genus-level groups in what I knew as the Iguanidae have gone through a number of

confusing changes (Etheridge 1964; de Queiroz 1987; Etheridge and de Queiroz 1988; Wiens 1993; Reeder 1995; Reeder, Wiens 1996; Macey et al. 1997; and Schulte et al. 2003). Schwenk (1994) discusses the underlying issues relating to nomenclature as classification and taxonomy have attempted to identify natural groups through cladistic analyses. Here, I follow Schulte et al. (2003), where Iguanidae, the "sceloporines" – now the subfamily Phrynosomatinae, and *Sceloporus* – including the species of *Sator* are all considered to be monophyletic.

- [2] Additional karyological data available for *Sceloporus* from publications subsequent to the Sites et al. (1992) review are summarized in current updates to Table 1 in Hall ([1977]), corresponding to the summary Table 4 in Sites et al. Unpublished data derived from my surviving records for other Iguanids and other families are also tabulated on - <http://tinyurl.com/vd2ta45>.
- [3] In agreement with Sites et al. (1992), clades diagnosed only by an increase or decrease in microchromosome numbers are weakly grounded, given difficulties resolving the internal morphologies of these very small objects. It is only an assumption that each decrease in microchromosome number happened only once.
- [4] This assumes that the disjunct F5 samples have a single derivation. Intermediate locations along the western interior of the Plateau where *grammicus* are known to occur from museum records (Fig. 10) have been poorly sampled for karyology.
- [5] Hall and Selander (1973) and Hall (1973) assumed that P1/HS was found only on the eastern side of the Valley of Mexico. This supported the idea that it might have differentiated recently. However, additional localities reported by Sites and Davis (1989) and Arévalo (1993) refutes the idea of recent derivation. Note that the contour shading shown on Sites and Davis (1989) Fig. 2 is mislabeled. From lightest to darkest the shades are below 2000 m, 2000-3000m, 3000-4000m, and above 4000m.

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