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Chromosome Evolution and Diversification in North American Spiny Lizards (Genus *Sceloporus*)

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Bayesian analysis · Character evolution · Genome · Phrynosomatidae · Speciation

Abstract
The genus *Sceloporus* is a diverse clade of lizards that exhibits substantial variation in chromosome numbers and sex chromosome heteromorphisms, 2 features of the genome that are static among most other pleurodont iguanian lizards. Evolutionary changes to the fundamental number of chromosomes are hypothesized to be a primary factor responsible for driving the diversification of *Sceloporus*. We explore the patterns of chromosome evolution in *Sceloporus* using a combination of ancestral state estimations and species diversification tests. Phylogenetic relationships and divergence times within *Sceloporus* (53 species representing all 19 species groups) are estimated using 4 nuclear genes (>3.3 kb) and relaxed-clock analyses that incorporate a fossil calibration on the root of the tree. We test the hypothesis that chromosome evolution is correlated with shifts in species diversification using cross-validation predictive densities, a new Bayesian approach for modeling the number of species that are predicted to have evolved in the absence of a certain historical event (e.g., a change in chromosome numbers). Results of the cross-validation predictive densities approach indicate that chromosomal evolution is correlated with significantly higher species diversity than predicted under the background rate of diversification in *Sceloporus*. We conclude by discussing the future of comparative cytogenetic investigations in *Sceloporus*. Copyright © 2010 S. Karger AG, Basel

The genus *Sceloporus* is an extremely diverse clade of lizards that has attracted the attention of evolutionary biologists and ecologists for decades [reviewed by Sites et al., 1992]. Factors contributing to the prominence of *Sceloporus* in comparative studies in evolution and ecology include (1) the genus is distributed broadly across North America and contains over 90 species, (2) *Sceloporus* contains some of the most abundant, conspicuous, and easily observable diurnal species, (3) *Sceloporus* exhibits high levels of variation in morphology, color patterns, behavior, ecology, life history, sexual dichromatism, and chromosome numbers, and (4) species-level phylogenetic trees are available for conducting comparative studies in a phylogenetic context.

The phylogenetic relationships among the major species groups of *Sceloporus* have changed radically since the

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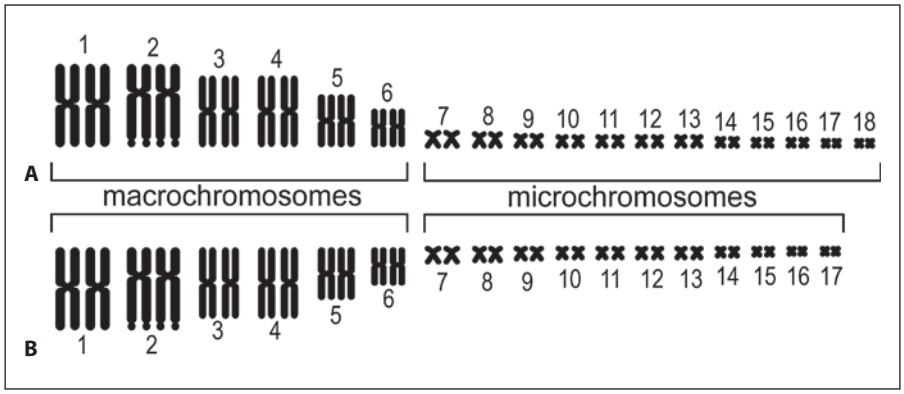

Fig. 1. A The ancestral 2n = 36 (12M + 22m + XY) karyotype for iguanian lizards is characterized by 6 pairs of bi-armed macrochromosomes and 12 pairs of microchromosomes (two of which are the sex chromosomes). **B** The ancestral 2n = 34 (12M + 20m + XY) phrynosomatid karyotype that is found in several of the basal lineages of *Sceloporus* differs from the standard iguanian karyotype by the loss of a single pair of microchromosomes. The secondary constrictions in the long arms of chromosome pair 2 represent the nucleolar organizing regions (NORs).

initial systematic studies conducted by Smith [1939] and Smith and Taylor [1950]. These first studies hypothesized that an early dichotomy separated the large-bodied, large-scaled species from the small-bodied, small-scaled species. Later investigations using phenetic clustering methods also supported a basal split in the genus [Larsen and Tanner, 1974, 1975], but the relationships among species groups changed dramatically. Although new hypotheses for the interrelationships among species groups continue to be proposed, the phylogenetic positions of some species groups are beginning to garner support from multiple, independent data sets. For instance, the basal division of *Sceloporus* into 2 major clades based on body and scale size is no longer supported. Instead, the small-bodied, small-scaled species form a paraphyletic group at the base of the phylogeny, and the *variabilis* group is the sister taxon to the remaining species groups [Wiens and Reeder, 1997; Flores-Villela et al., 2000; Leaché, 2010; Wiens et al., 2010].

Sceloporus is hypothesized to have undergone a rapid radiation – a period of increased rate of diversification – at some point in their evolutionary history [Mindell et al., 1989; Wiens and Reeder, 1997; Leaché, 2010]. Some of the difficulty in estimating phylogenetic relationships among species groups is attributable to the short time intervals that appear to separate some divergence events [Leaché, 2010]. As a whole, phrynosomatids (the clade containing *Sceloporus*) exhibit elevated levels of diversification [Harmon et al., 2003], the timing of which may correspond to the rapid radiation that took place within *Sceloporus*. Differentiation in the fundamental number of chromosomes among species and species groups is hypothesized to be a primary factor responsible for driving the rapid radiation of *Sceloporus* [Hall, 1973, 1980, 1983].

The ancestral karyotype for pleurodont iguanian lizards (which includes *Sceloporus*) is remarkably invariant, as revealed by conventional staining (aceto-orcein or Giemsa) [Gorman et al., 1967; Paull et al., 1976; Bickham, 1984]. The ancestral karyotype is 2n = 36, and is characterized by 6 pairs of bi-armed macrochromosomes and 12 pairs of microchromosomes, sometimes including distinct heteromorphic sex chromosomes. This can be written out in the following shorthand: 12M + 22m + XY, and is illustrated in figure 1A. The genus *Sceloporus* belongs to a more exclusive clade within the pleurodont iguanians, the phrynosomatids, and the ancestral karyotype of this group is hypothesized to be a 2n = 34 (12M + 20m + XY) arrangement (fig. 1B), differing by the loss of a single pair of microchromosomes [Hall, 1973; Paull et al., 1976]. The 2n = 34 karyotype may vary slightly if a different sex chromosome heteromorphism is present (see below), and the 2n varies extensively within *Sceloporus*, with diploid numbers ranging from a low of 2n = 22 to a high of 2n = 46 (fig. 2) [Sites et al., 1992].

Early models of chromosome evolution in *Sceloporus* emphasized linear increases or decreases in the number of chromosomes, and in most cases species with similar karyotype formulas were grouped together [reviewed by Sites et al., 1992]. The phylogenetic relationships within *Sceloporus* have changed substantially since these patterns of chromosome evolution were established, which motivates a reanalysis of chromosome changes on a modern phylogeny. We explore the patterns of chromosome evolution in *Sceloporus* using a combination of ancestral state estimations and species diversification tests. We estimate phylogenetic relationships and divergence times within *Sceloporus* using a previously published data set containing 53 species (representing all species groups) and 4 nuclear genes [Leaché, 2010]. We test the hypoth-

esis that chromosome evolution is correlated with shifts in species diversification using cross-validation predictive densities [Moore and Donoghue, 2009], a new Bayesian approach for modeling the number of species that are predicted to have evolved in the absence of a certain historical event (e.g., a change in chromosome numbers).

History of Chromosome Research

The earliest papers on chromosomal variation and evolution in the genus *Sceloporus* presented mitotic karyotypes visualized by conventional staining, and focused primarily on relationships within species groups native to the desert regions of the southwestern US and western Mexico [Hall, 1965; Lowe et al., 1967; Cole and Lowe, 1968; Cole, 1970, 1971a, b, 1972, 1975, 1977; Jackson and Hunsaker, 1970]. Subsequent studies by Hall [1973, 1980, 1983] extended to the entire genus, and also focused on the diverse chromosome races of the *Sceloporus grammicus* complex [see also Hall and Selander, 1973]. Hall's studies, and follow-up studies by one of us (J.W.S., with collaborators), emphasized testing various components of chromosomal speciation hypotheses [reviewed in Sites and Moritz, 1987; Sites et al., 1987; Sites, 1993; Sites and Reed, 1994; Marshall et al., 2006], and summarized patterns of karyotype variation in the genus [Sites et al., 1992].

In some *Sceloporus* karyotypes secondary constrictions are visible in the long arms of chromosome pair 2, which silver-staining techniques reveal to be the nucleolar organizing regions (NORs; sites of the ribosomal DNA repeats) in the 2n = 36 race (the F5 + 6 race of Hall [1973], fixed for fissions of macrochromosome pairs 5 and 6) of the *S. grammicus* complex [Sites, 1983]. The NOR regions may not be resolved by silver-staining if they are inactivated, and in a follow-up study Porter et al. [1991] hybridized a biotin-labeled probe (part of the *Mus* rDNA sequence) to visualize the location of the NORs by fluorescence microscopy. Their results confirmed that a single pair of NORs is present on the long arm of chromosome pair 2 across several species of *Sceloporus* representing a range of diploid numbers, including *S. undulatus* (2n = 22), *S. magister* (2n = 26), *S. graciosus* (2n = 30), *S. grammicus* (the 'standard' or S race of Hall [1973], 12M + 18m, X_1X_2Y) and *S. anahuacus* (the 'polymorphic 1' or P1 race of Hall [1973], variable for a fission of macrochromosome pair 1, but with X_1X_2Y), and *S. palaciosi* (some populations of the 'fission pair 6' or F6 race of Hall [1973]; fixed for a macrochromosome pair 6 fission, and also with X_1X_2Y). By contrast, Porter et al. [1991] report that in closely related genera *(Phrynosoma, Uta)*, which share the presumably ancestral karyotype (2n = 34, 12M + 20m + XY), the NOR pair fluoresces on a pair of microchromosomes, while in 2 other genera with the 'same' karyotype *(Cophosaurus, Holbrookia)*, the NORs are on pair 3 or 4. Thus, closely related genera possessing the same ancestral karyotype (as revealed by conventional staining) show non-identical patterns of NOR distribution. Clearly, some small chromosomal rearrangements have taken place among the genera, but cannot be detected with conventional staining methods.

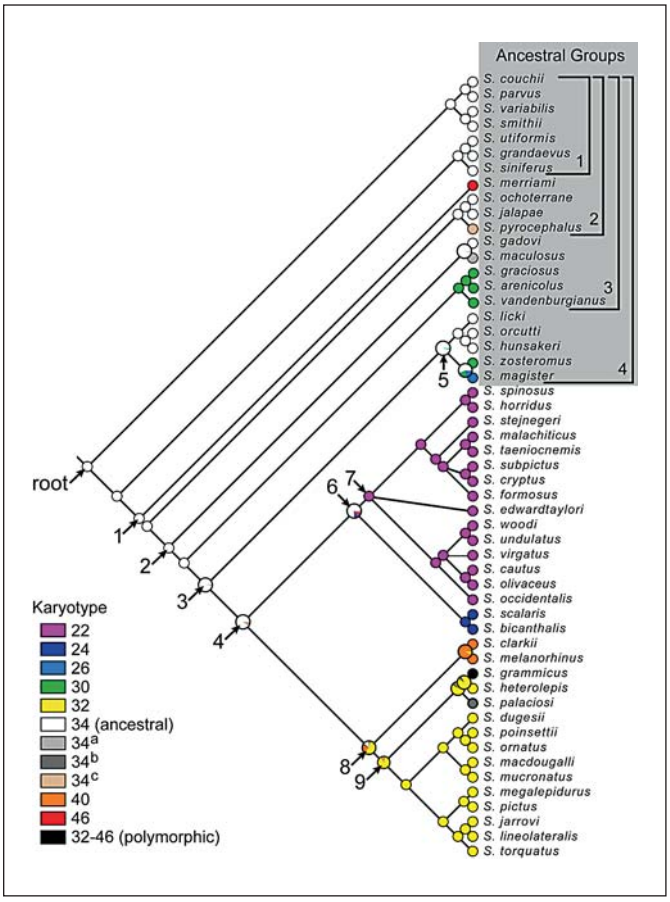

Fig. 2. Chromosome evolution in *Sceloporus*. Ancestral states are estimated using the MK-1 model in Mesquite. The phylogeny was estimated using a partitioned Bayesian analysis of 4 nuclear loci in BEAST. The 4 ancestral groups used to calculate background diversification rates are indicated. Bayesian ancestral state estimates for the numbered nodes are provided in table 2. Species possessing derived conditions of the ancestral karyotype number (2n = 34; i.e., *S. maculosus, S. palaciosi,* and *S. pyrocephalus*) are coded separately.

Efforts at higher resolution banding of *Sceloporus* karyotypes have, despite determined efforts by one of us (J.W.S.), either failed under a variety of conditions (G-banding), or revealed only small amounts of heterochromatin concentrated in centromeric regions [J.W.S., unpublished data]. However, early studies of chromosomal pairing behavior during meiosis (in males) [Porter and Sites, 1985, 1987] have provided indirect evidence of arm homology within and across different chromosome races of the *S. grammicus* complex (which range from 2n = 32 to 2n = 46) [summarized in Arévalo et al., 1991; Marshall et al., 2006]. These data have permitted the coding of chromosomal genotypes with some confidence for population cytogenetic studies [Porter and Sites, 1986; Arévalo et al., 1991], estimates of hybrid zone structure [Hall, 1973; Hall and Selander, 1973; Sites et al., 1993, 1995; Arévalo et al., 1993; Marshall and Sites, 2001; Leaché and Cole, 2007], estimates of fitness correlates in hybrid zones [Reed and Sites, 1995; Reed et al., 1995a, b; Sites et al., 1995], and phylogenetic studies [Wiens and Reeder, 1997; Flores-Villela et al., 2000] in the absence of high-resolution banding or fluorescent in situ hybridization studies.

Silver-staining studies of synaptonemal complex formation in males of several species of *Sceloporus* have provided details of the earliest stages of meiotic pairing, heretofore unattainable by conventional methods [Reed et al., 1990, 1992a]. Silver-staining has been successfully applied to study the pairing, recombination, and segregation dynamics for a within-race (2n = 34) pericentric inversion polymorphism [Reed et al., 1992b] and 2 rearrangements needed to derive chromosome pair 2 in the most derived race (2n = 46) of the *S. grammicus* complex [Reed et al., 1992c].

Sex Chromosome Diversity

Sceloporus is an ideal group in which to study the evolution of sex chromosome heteromorphisms. The genus is characterized by multiple, independently derived XY systems, all species are dioecious (no parthenogenesis is known), and sex is assumed to be controlled by genetic mechanisms because no temperature-controlled sex determination is known [Sites et al., 1992]. Not all species have morphologically differentiated sex chromosomes, but in those that do, males are always the heterogametic sex, which facilitates high-resolution meiotic studies [Reed et al., 1990]. It is possible that all species possess differentiated sex chromosomes and that the low resolving power of the cytogenetic methods may simply produce an observational bias. Further, the $X_1X_2Y♂$ heteromorphisms characterizing several species groups should not be taken as evidence of common ancestry (e.g., as in the *grammicus* and *torquatus* vs. the *maculosus* groups); these may represent independently evolved systems as well.

Hall [1973] considered the XY system with a minute Y chromosome ancestral for *Sceloporus*, on the basis of the presence of this system in the presumed sister genus *Uta* [Pennock et al., 1969]. Within *Sceloporus*, the minute Y is well documented in the *S. chrysostictus*, *S. merriami*, *S. utiformis*, and *S. variabilis* groups, and all of these but *S. merriami* are recovered as basal groups within *Sceloporus* on the basis of molecular data [Wiens and Reeder, 1997; Flores-Villela et al., 2000; Leaché, 2010; Wiens et al., 2010], providing independent evidence for Hall's hypothesis. Elsewhere in the genus, XX♀/XY♂ systems are known with 'uncertain Y morphology', and multiple sex chromosome heteromorphisms ($X_1X_1X_2X_2♀/X_1X_2Y♂$), derived by a likely Y-autosomal fusion [Cole and Lowe, 1967], appear to have had at least 3 independent origins. Some others include 'XY indistinct' [Cole, 1975], and 'XY indistinct except for enlarged pair 7' systems [summarized in table 5 of Sites et al., 1992]. One important highlight is that Hall [1973, 1980] suggested that the $X_1X_1X_2X_2♀/X_1X_2Y♂$ heteromorphism in the 2n = 31♂/32♀ karyotype was a synapomorphy uniting the large-sized, large-scaled 'crevice-user' clade (the *S. asper*, *S. grammicus*, *S. megalepidurus*, and *S. torquatus* groups).

Methods

Phylogeny and Divergence Times

We conducted phylogenetic analyses of *Sceloporus* to explore patterns of character evolution and to estimate diversification rates and divergence times. The data matrix used to perform phylogenetic analyses, published by Leaché [2010], contains representatives from each species group (53 species in 19 species groups) and 4 nuclear coding exons (*BDNF, PNN, R35,* and *RAG-1;* >3.3 kb). We conducted phylogenetic analyses using a relaxed molecular clock in BEAST v1.5.2 [Drummond and Rambaut, 2007]. These analyses utilized the same partitioning strategy (12 partitions, by gene and by codon position) as presented in Leaché [2010].

We inferred divergence times by assigning a fossil calibration to the root of the tree. *Sceloporus* are present in the fossil record by the early Miocene [Holman, 1970, 1995; Robinson and Van Devender, 1973; Yatkola, 1976], and uncertainty in this fossil calibration was incorporated into the divergence dating analysis using a lognormal prior distribution on the root node (offset = 25 mya; standard deviation = 1.0). Substitution rate variation among lineages was modeled using an uncorrelated lognormal distribution. We ran the analysis under 2 different priors for the branching process, including a Yule (pure birth) and a birth-death prior. We enforced our preferred rooting of the phylogeny (the *variabilis* group) by defining all remaining species as a monophyletic

Table 1. Karyotype diversity of the monophyletic species groups of *Sceloporus*

Species group	Species	2n	Sex chromosomes
angustus	2	34	XX♀/XY♂
clarkii	2	40	XX♀XY♂, $X_1X_1X_2X_2$♀/X_1X_2Y♂
edwardtaylori	1	22	indistinct
formosus	14	22	indistinct
gadoviae	1	34	XX♀/XY♂
graciosus	3	30	indistinct
grammicus	5	32–46	$X_1X_1X_2X_2$♀/X_1X_2Y♂
jalapae	2	34	XX♀/XY♂
maculosus	1	34[a]	$X_1X_1X_2X_2$♀/X_1X_2Y♂
magister	6	26/30/34	XX♀/XY♂, indistinct
merriami	1	46	XX♀/XY♂
pyrocephalus	2	34[b]	XX♀/XY♂
scalaris	8	24	XX♀/XY♂
siniferus	4	34	XX♀/XY♂
spinosus	2	22	indistinct
torquatus	18	32	$X_1X_1X_2X_2$♀/X_1X_2Y♂
undulatus	10	22	indistinct
utiformis	1	34	XX♀/XY♂
variabilis	6	34[c]	XX♀/XY♂

[a] Differs from ancestral state in the ratio of autosomes to sex chromosomes.
[b] Differs from ancestral state in the autosomal karyotype formula.
[c] Secondary constriction adjacent to the centromere on the largest pair of microchromosomes.

group after observing that the BEAST analysis was estimating a root position that was in conflict with pervious studies [Wiens and Reeder, 1997; Flores-Villela et al., 2000; Leaché, 2010; Wiens et al., 2010]. The MCMC analyses were run for 10 million generations, logged every 1,000 steps, and the divergence times for nodes of interest were sampled at the same frequency. We used Tracer v 1.4.1 to determine convergence, measure the effective sample size of each parameter, and calculate the mean and 95% highest posterior density interval (HPD) for divergence times and diversification rate [Rambaut and Drummond, 2009].

Ancestral State Estimation

Karyotype data for *Sceloporus* were taken from Sites et al. [1992; and references therein], which provides a summary of the chromosomal data available since the 1970s. For the ancestral state estimations conducted in this study, we utilized 2 aspects of chromosome variation, including the fundamental chromosome number (the 2n number), and the morphology of the sex chromosomes. Chromosome number was treated as an unordered, multistate character with 12 states (table 1). Species with similar chromosome numbers that possess rearrangements or different combinations of autosomes to sex chromosomes were coded with distinct character states (i.e., *S. maculosus, S. palaciosi,* and *S. pyrocephalus*). Sex chromosomes were coded using 6 unordered character states [reviewed by Sites et al., 1992], including 4 character states for the XX♀/XY♂ system (1, minute Y; 2, large, acrocentric Y; 3, sex chromosomes indistinct or missing; and 4, chromosome pair 7 heteromorphism) and 2 character states for the $X_1X_1X_2X_2$♀/X_1X_2Y♂ system (1, fusion of ancestral Y chromosomes onto acrocentric microchromosome; 2, fusion of ancestral Y chromosomes onto acrocentric pair 5 fission product).

Estimates of ancestral karyotype numbers that take advantage of stochastic models of evolution were inferred using maximum likelihood (ML) [Pagel, 1999] and Bayesian inference [Pagel et al., 2004]. The ML estimate assigns to the ancestral nodes character states that maximize the likelihood of the observed data for the terminal taxa, given a model of evolution. The model of evolution is the Markov k-state 1 parameter model (Mk1 model), which contains a single parameter for the rate of change among any character state [Lewis, 2001]. Ancestral character states were inferred using the Mk1 model in Mesquite v.2.5 [Maddison and Maddison, 2009]. The maximum clade credibility tree that resulted from the BEAST analysis was used for character mapping. Bayesian inference provides estimates of the marginal posterior probability of ancestral states using model-averaging [Pagel et al., 2004]. Reversible-jump Markov chain Monte Carlo (RJMCMC) is used to integrate over all plausible models, which relaxes the constraint of conditioning inferences on a single model of trait evolution [Pagel and Meade, 2006]. Advantages of the Bayesian approach are that it averages over all k-state models of character evolution and accommodates uncertainty in the phylogeny and the rate parameters of the models [Pagel and Meade, 2006]. Bayesian inference of ancestral character states was conducted using the posterior distribution of phylogenies obtained from a MrBayes analysis [see Leaché, 2010 for details] using the program BayesTraits v1.0 [Pagel and Meade, 2009]. We did not utilize the posterior distribution of

trees from BEAST, because that analysis included a topological constraint that removed uncertainty in the root position. The posterior probability distributions from 4 independent RJMCMC analyses, each run for 2 million generations (burn-in and sample period = 100,000), were compared to ensure convergence and combined to produce final estimates of the probabilities for ancestral state estimates.

Exploring the Association of Chromosomal Evolution with Diversification

To determine if chromosomal evolution was significantly associated with shifts in rates of diversification in *Sceloporus,* we used cross-validation predictive densities [Moore and Donoghue, 2009]. This Bayesian approach seeks to determine if shifts in species diversification rate are associated with historical events on a phylogeny. The method produces a predictive distribution of species diversity that would be expected if the historical event had not occurred (i.e., a predictive diversity distribution). The observed species diversity associated with the event is compared against the predictive distribution to assess whether rates of diversification are significantly higher or lower than expected [Moore and Donoghue, 2009]. The predictive diversity distribution is calculated using the marginal posterior probability distributions for (1) the divergence time for the clade of interest, and (2) the background diversification rate for the taxa not associated with the event [Moore and Donoghue, 2009].

Evolutionary changes in chromosome number occur throughout the history of *Sceloporus* [Sites et al., 1992], making it difficult to associate one clade with a pivotal historical event where chromosome evolution first occurred. Therefore, we selected 4 nodes in the *Sceloporus* phylogeny to test the hypothesis that chromosome evolution is correlated with shifts in species diversification rate. These nodes span the backbone of the *Sceloporus* phylogeny and were selected after interpreting the results of the ancestral state estimation. The background diversification rates are calculated by conducting separate BEAST analyses with the clade of interest excluded from the analysis. Background diversification rates were then calculated for the following collections of ancestral species groups in *Sceloporus* (see fig. 2):

Ancestral Group 1 – *variabilis, angustus, utiformis, siniferus* groups.

Ancestral Group 2 – *merriami, pyrocephalus, jalapae* groups, and Ancestral Group 1.

Ancestral Group 3 – *graciosus, gadoviae, maculosus* groups, and Ancestral Group 2.

Ancestral Group 4 – *magister* group, and Ancestral Group 3.

We performed the cross-validation predictive density simulation (1 million simulations) and calculated posterior predictive p-values using the program tRate [Moore and Donoghue, 2009]. Diversification rates will be underestimated when taxon sampling is incomplete, as is the case with the current *Sceloporus* data matrix. To obtain more accurate estimates for the background diversification rates, we conducted sensitivity analyses whereby we added missing species into the BEAST analyses by duplicating the sequences available for closely related members of the same species group. For these tests, we also doubled the divergence times for the clades of interest to conduct a more conservative test of the diversification hypothesis.

Results

Chromosomal Evolution

The earliest instance of an evolutionary change in the number of chromosomes among phrynosomatid lizards from the ancestral state of 2n = 34 occurs along the branch leading to *S. merriami,* which has a highly derived karyotype of 2n = 46 (fig. 2, 3). The fission of 6 macrochromosomes is hypothesized to have resulted in this increase in chromosome number [Cole, 1971a]. Several lineages of *Sceloporus* diverge while retaining the ancestral karyotype before the next event, in which the fusion of 2 microchromosomes results in the 2n = 30 karyotype shared among species in the *graciosus* group (fig. 2, 3). Within the *magister* group, *S. orcutti, S. licki,* and *S. hunsakeri* all retain the ancestral 2n = 34, while *S. zosteromus* and *S. magister* have undergone a series of microchromosomal fusions resulting in karyotypes of 2n = 30 and 2n = 26, respectively (fig. 2, 3). Data quality may be an issue for the *S. zosteromus* karyotype reported to date [Hall, pers. comm.], so published results require further verification.

The remaining features of chromosome evolution in *Sceloporus* include 2 components, one of which is characterized by a reduction in the number of chromosomes, presumably via a series of microchromosome fusions (fig. 2, 3). One clade is the *scalaris* group (2n = 24), and it is recovered as the sister group to a large clade that includes the *undulatus*, *edwardtaylori*, *spinosus*, and *formosus* groups, each of which has 22 chromosomes (fig. 2, 3). The second component is characterized by both increases and decreases in chromosome numbers. The apparent fission of 4 macrochromosomes results in 40 chromosomes in the common ancestor of the *clarkii* group (fig. 2, 3). An inferred fusion of 1 microchromosome pair results in a 32-chromosome clade containing the *torquatus* group (fig. 2, 3). Finally, the *grammicus* group contains species and/or populations of *S. grammicus* with karyotypes ranging from 2n = 31♂/32♀ to 2n = 45♂/46♀ (fig. 2, 3).

Sex Chromosome Evolution

Hall's hypothesis [1973] that the XX♀/XY♂ system with a minute Y chromosome is ancestral for *Sceloporus* is supported by the data presented here (fig. 4). At least 9 changes to the sex chromosomes are inferred in *Sceloporus*, and some of these changes include independent derivations of similar appearing sex chromosome systems (on the basis of conventional staining; fig. 4). The XX♀/XY♂ system is indistinct or missing in at least 3 separate clades, which include the *graciosus* group, the *magister* group, and the clade containing the *scalaris* group and

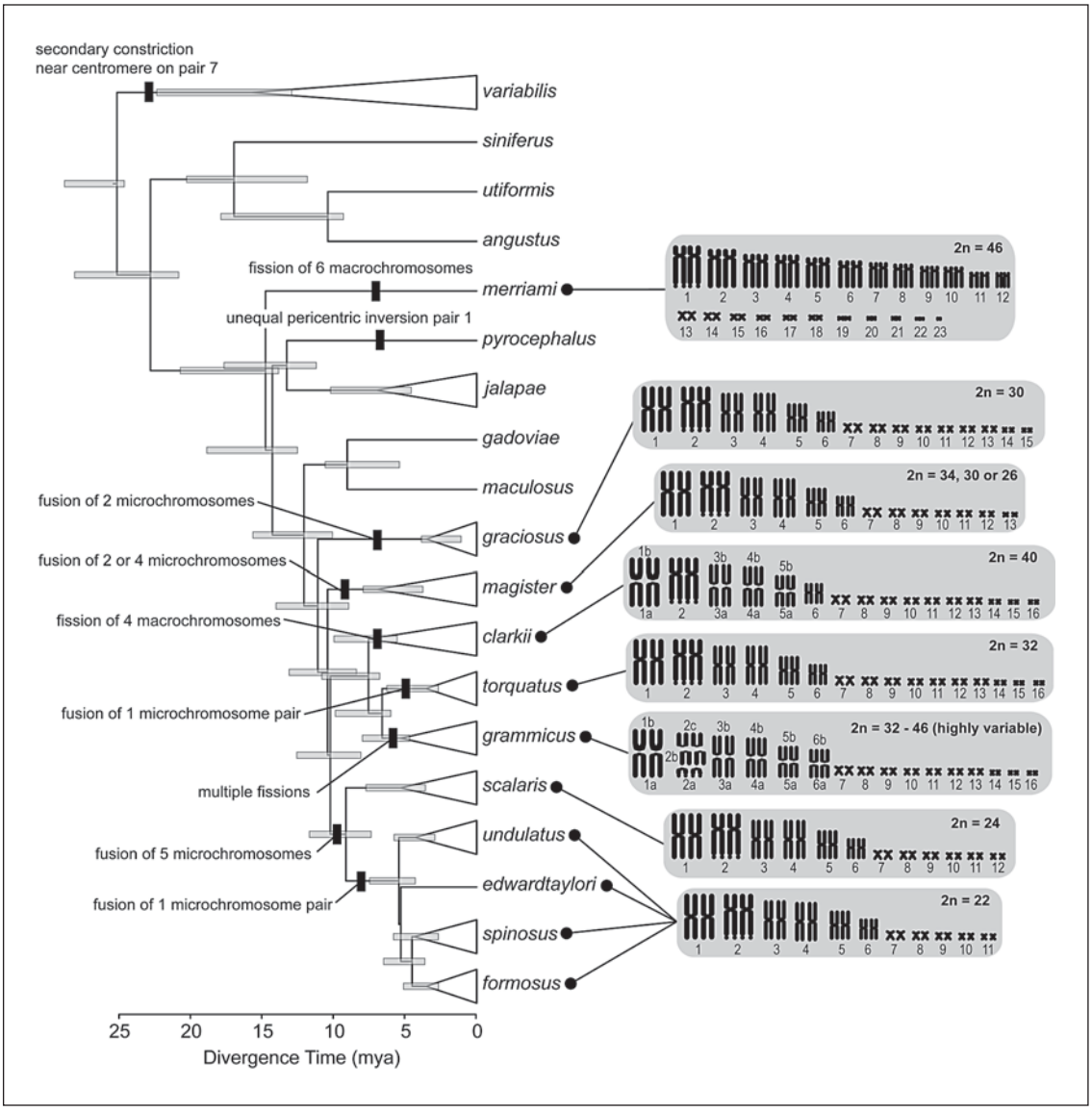

Fig. 3. Chronogram of divergence times among the monophyletic species groups of *Sceloporus* inferred from a BEAST analysis assuming a birth-death tree prior. Evolutionary changes in chromosome structure are mapped on the tree, and the modifications producing karyotype numbers that are distinctive from the ancestral 2n = 34 are shown.

the 22-chromosome clade (i.e., *edwardtaylori, formosus, spinosus,* and *undulatus* groups; fig. 3). Although the sex chromosomes in these species are not distinguishable using standard Giemsa-staining techniques, there is no evidence of environmentally determined sex in any *Sceloporus,* and it is reasonable to assume that sex is genetically-based on an XY system. These assessments are a potential underestimate of the number of independent gains/losses of this character, since some of the species belonging to these clades appear to retain a visible XX♀/XY♂ system [Sites et al., 1992].

The 22-chromosome clade is also unique in that it contains species that exhibit a chromosome pair 7 heteromorphism (fig. 4), which results in variation in the location of the centromere along the chromosome [Cole, 1970]. In at least 1 species, *S. lundelli*, chromosome pair 7 is heteromorphic and reported to be the sex chromosome pair [Cole, 1970]. Several species with broad distribu-

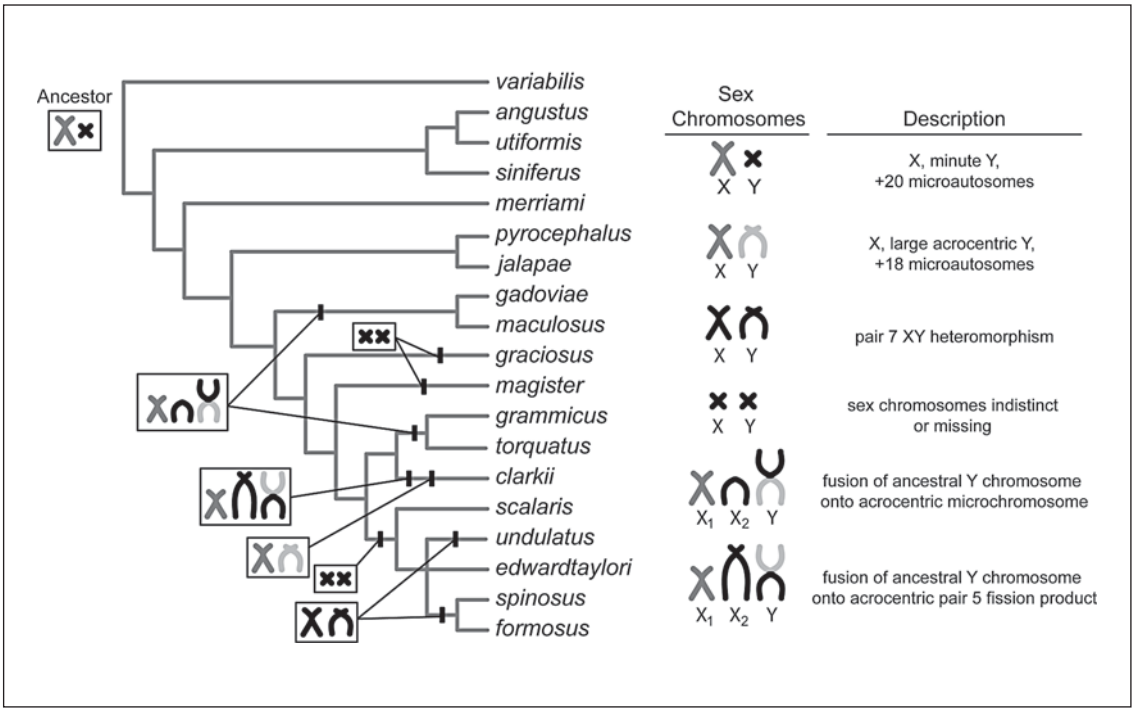

Fig. 4. Sex chromosome evolution in *Sceloporus*. The shading of the chromosomes indicates presumed homology [adapted from Sites et al., 1992]. The phylogeny is based on a BEAST analysis assuming a birth-death tree prior.

Table 2. Marginal posterior probability distributions for ancestral karyotype estimates in *Sceloporus* obtained using BayesTraits

Node	Karyotype (2n)											
	22	24	26	30	32	34	34[a]	34[b]	34[c]	40	46	32–46
Root	–	0.02	0.02	0.07	–	**0.78**	0.02	0.02	0.02	0.01	0.02	0.01
1	0.02	0.05	0.05	**0.28**	0.02	0.13	0.05	0.07	0.04	0.05	0.20	0.04
2	0.01	0.06	0.04	**0.60**	0.02	0.08	0.02	0.01	0.03	0.09	0.01	0.03
3	0.04	0.15	0.06	**0.21**	0.07	0.09	0.04	0.02	0.03	**0.21**	0.02	0.05
4	0.10	0.21	0.03	0.04	0.14	–	0.05	0.02	0.02	**0.31**	0.02	0.05
5	–	0.01	0.09	0.37	0.00	**0.43**	0.01	0.01	0.02	0.01	0.01	0.02
6	0.36	**0.41**	0.03	0.04	0.01	–	0.02	0.02	0.02	0.03	0.03	0.02
7	**1.00**	–	–	–	–	–	–	–	–	–	–	–
8	–	0.01	0.01	0.02	0.30	–	0.08	0.01	0.01	**0.48**	0.01	0.06
9	–	0.01	0.01	0.01	**0.71**	–	0.14	0.01	0.01	0.02	0.01	0.08

The character state receiving the highest posterior probability is shown in bold for each node mapped on the phylogeny in figure 2.
[a] Differs from ancestral state in the autosomal formula and sex chromosomes *(Sceloporus palaciosi)*.
[b] Differs from ancestral state in the autosomal karyotype formula *(Sceloporus pyrocephalus)*.
[c] Differs from ancestral state in the ratio of autosomes to sex chromosomes *(Sceloporus maculosus)*.

Table 3. Cross-validation predictive density results indicate that species diversity in *Sceloporus* is significantly higher than predicted under background diversification rates

Historical event	Background diversification rate		Divergence time	p	p^{Alt}
	Yule model	birth-death process			
Ancestral Group 1/Clade 1	0.0546 (0.016–0.096)	0.0459 (0.007–0.090)	19.59 (15.06–24.21)	<0.001	0.338
Ancestral Group 2/Clade 2	0.0577 (0.025–0.095)	0.05 (0.046–0.091)	14.05 (10.94–17.75)	<0.001	0.033
Ancestral Group 3/Clade 3	0.0681 (0.034–0.105)	0.0607 (0.022–0.101)	11.84 (9.09–14.95)	<0.001	0.007
Ancestral Group 4/Clade 4	0.0811 (0.046–0.122)	0.0725 (0.03–0.12)	11.29 (8.66–14.12)	<0.001	0.018

The locations of the historical events on the phylogeny are illustrated in figure 2. The divergence time estimates and p values shown are from the analyses assuming a Yule model for the tree prior. The results of the analyses using the birth-death tree prior are all significant (p and $p^{Alt} < 0.05$; results not shown). Figures in parentheses represent 95% credible intervals.

p^{Alt} is the significance for a conservative model that assumes full taxon sampling for the ancestral group and a 2-fold increase in the divergence time of the clade of interest.

tions, including some species in the *undulatus* group, exhibit geographic variation in the chromosome 7 heteromorphism [Cole, 1972; Leaché and Cole, 2007]. The chromosome 7 heteromorphism appears to be a highly labile character that is present in many species belonging to the 22-chromosome clade.

Multiple sex chromosome heteromorphisms ($X_1X_1X_2X_2$♀/X_1X_2Y♂), derived by a likely Y-autosomal fusion [Cole and Lowe, 1967], appear to have had 3 independent origins (fig. 4). As suggested by Hall [1973, 1980], the $X_1X_1X_2X_2$♀/X_1X_2Y♂ heteromorphism in the 2n = 31♂/32♀ karyotype is a synapomorphy uniting the large-sized, large-scaled 'crevice-user' clade (i.e., the *grammicus* and *torquatus* groups), and this sex chromosome system has a second independent derivation in the *maculosus* group (fig. 4). Lastly, a unique $X_1X_1X_2X_2$♀/X_1X_2Y♂ system and XX♀/XY♂ appear to have evolved in the *clarkii* group in *S. melanorhinus* and *S. clarkii,* respectively (fig. 4).

Ancestral State Estimates

Estimating ancestral karyotype numbers using maximum likelihood (ML) or Bayesian inference suggests that the ancestral 2n = 34 karyotype is the most likely character state for the most recent common ancestor of *Sceloporus* (fig. 2, table 2). For the ML analysis, all ancestral nodes up to and including clade 6 are inferred to retain the ancestral state (fig. 2). However, the Bayesian analysis is much less decisive, and the posterior probabilities of ancestral states at these same nodes are generally much lower (posterior probability values ≤0.60; table 2).

The ML and Bayesian methods both provide strong support (posterior probability = 1.0) for the ancestral state estimate of the 22-chromosome clade, which has an inferred state of 22 chromosomes (node 7; fig. 2, table 2). The Bayesian analysis is somewhat equivocal as to whether the most recent common ancestor of the 22-chromosome clade + the *scalaris* group (node 6; fig. 2) is composed of 22 chromosomes (posterior probability = 0.36) or 24 chromosomes (posterior probability = 0.41). The ML analysis suggests that the ancestral karyotype (2n = 34, 12M + 22m) is most likely for this clade (fig. 2). The posterior probability for the ancestral state estimate of the 32-chromosome clade is high (posterior probability = 0.71, node 9; table 2), and the ML analysis supports this result as well (fig. 2). The next most inclusive node of the phylogeny, which includes the *clarkii* group (node 8), receives a posterior probability of 0.48 for 40 chromosomes from the Bayesian analysis, while the ML analysis supports a 32-chromosome ancestor for this clade (fig. 2).

Exploring the Association of Chromosomal Evolution and Diversification Rates

The results of the cross-validation predictive densities approach suggest that chromosomal evolution is correlated with increased rates of diversification in *Sceloporus*

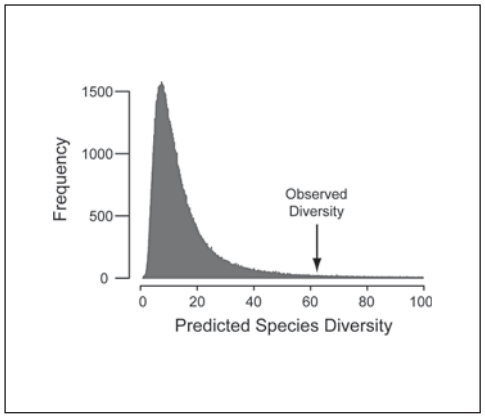

Fig. 5. Predicted species diversity for *Sceloporus* obtained from the cross-validation predictive densities approach. The observed number of species (n = 61) is significantly higher than predicted by the background diversification rate ($p < 0.018$). The distribution was estimated using the marginal posterior probability distributions for the rate of diversification of ancestral group 4 and the timing of the divergence event at clade 4 (fig. 2).

(table 3). The hypothesis that the observed species diversity is not significantly higher than expected under the background diversification rate is rejected for all 4 ancestral groups tested (table 3, fig. 5). These results are robust to the prior used to model the branching process (i.e., a Yule or birth-death prior; table 3). The marginal posterior estimates of the background diversification rate show a trend towards increasing for the more inclusive ancestral groups (table 3) which suggests that species diversification rates in *Sceloporus* may have increased through time. The timing of these events ranges from approximately 19.6 mya to as recent as 11.3 mya, but these estimates are accompanied by large credible intervals ranging from 24.2–8.6 mya (table 3). The cross-validation predictive density approach integrates over the marginal posterior probability distributions for background diversification rate and divergence time, and therefore accommodates the uncertainty associated with these parameters [Moore and Donoghue, 2009].

Under the more conservative tests of the hypothesis that chromosomal evolution is correlated with rates of diversification, which assume complete species sampling and a 2-fold increase in the divergence time for the clades of interest, the observed number of species is not significantly higher than predicted from the background diversification rate for Ancestral Group 1 under the Yule branching prior ($p^{Alt} = 0.338$; table 3). However, the cross-validation predictive density simulation results are significant for the remaining historical events under the conservative model and the Yule prior ($p^{Alt} \leq 0.033$; table 3). Under the birth-death prior, all results are significant (p and $p^{Alt} \leq 0.05$; results not shown).

Discussion

Chromosome Evolution and Species Diversification

It is becoming increasingly clear through continued phylogenetic investigations that the evolutionary history of *Sceloporus* includes periods of rapid radiation [Mindell et al., 1989; Wiens and Reeder, 1997; Leaché, 2010]. The short internodes occurring between subsequent divergence events deep in the *Sceloporus* phylogeny provide evidence for which species groups are a product of the rapid radiation, but the timing of the divergence events has remained unclear. The divergence dates estimated here suggest that *Sceloporus* clades associated with the rapid radiation may have diverged in the Miocene, ranging from 19.6 mya to as recent as 11.3 mya (table 3). Given that a relatively constrained prior distribution on the root node is the only calibration used to obtain these dates, they should be considered tentative. Future analyses utilizing multiple calibrations that encompass the root and the tips of the phylogeny should provide divergence time estimates with much narrower confidence intervals than those reported here. Regardless, the divergence dating analyses presented here provide a new chronology of divergence events within *Sceloporus* that place the rapid radiation onto a geological time-scale. Integrating the pre-existing karyotype data collected for *Sceloporus* with new phylogenetic data on a temporal scale allows us to test the long-standing hypothesis that chromosomal evolution is correlated with rates of diversification in *Sceloporus*.

In *Sceloporus,* increased rates of diversification are apparently correlated with episodes of chromosomal evolution. We conducted simulations to test this hypothesis at 4 nodes in the *Sceloporus* phylogeny, which include a broad set of divergence events spanning the period of the rapid radiation. In each instance, the observed number of species within *Sceloporus* is significantly higher than predicted based on background diversification rates (table 3), although simulations conducted under more conservative test conditions do not produce significant results for the oldest group tested when assuming a Yule model for the tree prior (Ancestral Group 1, table 3). The Bayesian technique used here relies on cross-validation predictive densities and has a number of attributes that

make it a robust inference framework [Moore and Donoghue, 2009]. The approach accommodates error terms associated with the divergence time estimates, which in the case of *Sceloporus* are quite high due to a paucity of reliable calibration points. In addition, uncertainty in the background diversification rate is also incorporated into the analysis.

Species diversification rates are not static within *Sceloporus*, but instead show a trend towards increasing as more inclusive ancestral groups are examined (table 3). This could be a product of the shape of the *Sceloporus* phylogeny, which is strongly pectinate at the base (fig. 2). More specifically, the sequential divergence of *Sceloporus* species groups from the base of the tree up to the divergence of the *magister* group results in an asymmetric tree, which then becomes relatively symmetrical for the 22-chromosome and 32-chromosome clades and their sister taxa (fig. 2). The symmetrical portion of the tree contains the most species-rich groups within *Sceloporus* and accounts for ~70% of all species in the genus. This group diverged relatively recently (8.66–14.12 mya), and includes many derived changes to the autosomes and the sex chromosomes that are unique among pleurodont iguanian lizards.

Another point of interest is that 3 distinct patterns of correlation between chromosomal evolution and diversification rates are evident within *Sceloporus*. At 3 nodes in the tree, diversification shows a pattern in which individual speciation events are associated with single (usually) autosomal chromosomal rearrangements (i.e., the *merriami*, *graciosus*, and *magister-zosteromus* groups; nodes 1, 2, and 5, respectively, in fig. 2). At 2 other nodes, either 1 (node 9, *S. torquatus* group) or several (node 7, *S. undulatus* group) autosomal rearrangements are fixed and define bursts of speciation with no further karyotypic change occurring within a clade. The single exception is the *S. grammicus* complex, which shows a third pattern consisting of extensive chromosomal polytypy within a single species or species complex (table 1) [Hall, 2009]. These distinct phylogenetic patterns suggest different kinds of evolutionary events associated with chromosomal evolution, even when the types of chromosomal rearrangements established are the same (i.e., Robertsonian fusions or fissions; table 1). The dominant patterns are that chromosomal evolution is either associated with individual speciation events, or with 'kick starting' a relatively rapid radiation, which is then characterized by increased species diversification with chromosomal stability (fig. 2).

Do Chromosomal Rearrangements Drive Speciation in Sceloporus?

Mechanisms that result in rapid radiations are intriguing because they are responsible for producing large numbers of species over short evolutionary periods. The hypothesis that chromosomal evolution may be causally linked to speciation in *Sceloporus* was established as early as in the 1960s when karyotypic variation was still being described in the genus [Hall, 1965; Lowe et al., 1967]. Later work by Hall [1973, 1980, 1983, 2009] focused heavily on detailing the models of chromosome speciation in the genus, in which fixations of chromosomal rearrangements in peripheral populations established the initial barriers to gene flow between populations [see also White, 1978a; King, 1993; Noor et al., 2001].

Specifically, these hypotheses were based on the following 5 assumptions or requirements: (1) in the original heterozygous state, chromosomal rearrangements are assumed to be underdominant; (2) underdominance derives from meiotic malassortment at the first division to produce a high proportion of aneuploid gametes; (3) chromosomal underdominance in turn requires a strongly 'Wrightian' demographic structure characterized by small population sizes, which will permit occasional fixation of rearrangements by stochastic processes (e.g., genetic drift); (4) fixation in a local deme is then followed by the spread of new homozygous genotypes, which upon secondary contact with the ancestral chromosome race forms a 'tension zone' [Key, 1968] in which chromosomal differences between hybridizing populations function as a partial post-mating reproductive isolating mechanism; and (5) F1 hybrids and possibly some backcross combinations are less fit than parentals on either side of the tension zone due to any number of meiotic anomalies [White, 1973], and the tension zone itself thus acts as a partial barrier to gene flow [see also Barton and Hewitt, 1991]. When this is the case, natural selection is then postulated to favor the evolution of pre-mating isolating mechanisms to reduce formation of hybrids – a type of 'reinforcement' that drives speciation to completion [Dobzhansky, 1951, 1970]. Various authors differed on whether 1 chromosome change [White, 1968] or several [Hall, 1980, 1983; Walsh, 1982; White 1978b] were required, but initially all models required genetic drift to establish new rearrangements [reviewed by Sites and Moritz, 1987; Sites and Reed, 1994].

These early models were based on observing that closely related species often differed in their karyotypes by the fixation of one or more rearrangements that were presumed to have been underdominant at their time of

origin as individual heterozygotes. Further, within-species cytogenetic surveys usually showed that intra-population polymorphisms for the kinds of rearrangements diagnostic of between-species differences were rare or absent [White, 1973]. These observations were taken together as primary evidence for the stochastic fixation of such rearrangements and their subsequent causal, or at least major, contributing role in species divergence.

King [1993] has argued most forcefully for an explicit causal role between the fixation of chromosomal rearrangements and the initiation of speciation, and suggested that meiotic drive was sufficiently well established to permit an alternative to the 'fixation by drift' requirement of earlier models. A review of that book by one of us [Sites, 1995] suggested that the premise for stochastic fixation of underdominant rearrangements in subdivided populations was not well supported, and that meiotic drive was not nearly so well documented as King had argued. A more recent review [Jaenike, 2001] reveals that the best documented examples of meiotic drive are in the sex chromosomes (also known as sex-ratio or segregation distortion) in some insects, mammals, and angiosperms [table 1 in Jaenike, 2001]. The issue of more relevance here is autosomal drive as argued by King and others, and this has been documented by QTL studies in model organisms, but many of these cases involve plant hybrids in which the apparent segregation distortion may be due simply to differential pollen inviability and/or fertilization success. At best, the mechanism of meiotic drive remains a very tenuous proposition for linking chromosomal rearrangements to the initiation of speciation events.

In the absence of convincing evidence for relevant forms of meiotic drive, we are left with some form of restrictive drift/fixation requirement, or establishment of rearrangements that may be adaptive via protection of coadapted gene combinations on the rearranged chromosomes [White, 1978b]. White's argument is based on the idea that local populations of most low vagility species will exhibit 'area effects' in which some geographic regions are characterized by allelic combinations that are rare/absent in other parts of the range. Under these conditions, any chromosomal rearrangement that 'locks up' area effect alleles into protected linkage groups [via recombination suppression, see Rieseberg, 2001] that will not be disrupted by gene flow from outside of the area would have a reasonable chance of fixation. In this example the novel rearrangement would have to spread until it coincided with the area effect, but it would not necessarily need to be strongly underdominant in the heterozygous state and thus not need to drift to fixation in a small population.

Hall [2009] suggests that some of the Robertsonian rearrangements that dominate chromosomal evolution in *Sceloporus* (centric fusions and fissions) may function in this manner, based on evidence of the number and location of chiasmata formation (scored from male diakinesis arrays) in the larger bi-armed macrochromosomes relative to the fission mutations. These and related points made by Hall in his contribution to this volume have been used to describe a 'cascading speciation model' based on an approximately linear derivation of chromosomal rearrangements, from origin to termination. We will not repeat the details of Hall's model here, but the idea is an intriguing one, and the *S. grammicus* complex figures prominently in its development. Two aspects of this model do seem to us to be empirically testable in this group because it is likely a very recent case of this cascade process, or at least an approximately linear derivation of the establishment of derived chromosome races from an ancestral 2n = 32 karyotype [Sites and Davis, 1989; Arevalo et al., 1994]. The derived races in this complex are restricted to a small area in the Valley of Mexico that has been dominated by dry-land agriculture for the past few thousand years [Hall, 2009]. If sequential colonization of these environments by the most derived chromosome races in the *S. grammicus* complex has been accompanied by substantial population expansions, as the cascade process suggests, then high-resolution molecular markers (e.g., SNPs or microsatellites) should detect a signature of successive 'waves' of population expansion. This is because rapid population growth alters the frequency distribution of alleles in predictable directions, relative to neutral population models at demographic equilibrium [Rogers and Harpending, 1992].

Second, if fixed chromosomal differences between hybridizing populations suppress recombination and protect co-adapted linkage groups, then loci close enough to the centromere to 'hitch-hike' along a non-recombining flanking region could accumulate alleles specific to each race, and the zone would then be 'semi-permeable' [Harrison, 1990] permitting some gene flow, but not those alleles in the protected linkage groups [Rieseberg et al., 1999]. Linkage-dependent gene flow has been demonstrated across a European house mouse *(Mus musculus)* chromosomal hybrid zone, in which races are distinguished by Robertsonian rearrangements (centromeric fusion/fission mutations) [Panithanarak et al., 2004], and the same phenomenon might operate in the *S. grammicus* complex. The chromosome races are defined by Robert-

sonian rearrangements, and one prediction of the 'recombination suppression' hypothesis is that genes linked to the centromeric region will reflect the same structure as the chromosomal differences that define the zone (i.e., very limited gene flow over very short differences) [Hall and Selander, 1973; Arévalo et al., 1993; Sites et al., 1993, 1995; Marshall and Sites, 2001], while genes in the recombining regions should move freely across the zones. There is no genetic map for this or any other species of *Sceloporus*, so unlike *Mus* the genomic resources are not presently available to implement this kind of study, but the predictions are very clear about what should happen in these semi-permeable barriers to gene flow.

Studies of the role of chromosomal evolution as a possible contributor to speciation will require multidisciplinary approaches to several interrelated issues, including internal molecular, cytomechanical, and meiotic processes associated with the origins of chromosomal rearrangements, as well as external issues of effective population size, gene flow, metapopulation structure and dynamics, hybrid zone dynamics, and fitness correlates of chromosomal heterozygosity in parental, hybrid, and backcross generations [Sites, 1983; Sites and Reed, 1994]. These kinds of studies need to be carried out in chromosomally polytypic taxa thought to be undergoing some form of chromosomally-mediated divergence [Hall, 1973, 2009; White, 1978a], and the chromosome races of the *S. grammicus* complex can continue to be one of several vertebrate models for these kinds of studies [reviewed in Marshall et al., 2006].

Future Research

Future comparative cytogenetic studies of *Sceloporus* will benefit tremendously from the availability of a more detailed database describing the variability of chromosome structures among species. The current descriptions of karyotypes obtained from traditional staining techniques are insufficient for describing the morphology of the microchromosomes in great detail, which leaves the description of some aspects of the chromosome morphology open to interpretation (i.e., centromere location). In other cases, for instance the presumed absence of the XY system in some species, current interpretations may be an artifact of the low-resolution visualization technique. Thus, the establishment of chromosomal homologies between different species should be an immediate goal for future cytogenetic and genomic studies in *Sceloporus*; presuming homology based on chromosome size and shape is inadequate, especially for the microchromosomes. High-resolution banding or fluorescent in situ hybridization techniques offer the potential to refine these predictions for all chromosomes and help identify the location of the sex chromosomes [Waters et al., 2009]. Laboratory protocols for blood or tissue (fibroblast cell) cultures, chromosome microdissection, and flow sorting of chromosomes by size, when coupled with genomic approaches to screen BAC libraries, and isolate candidate genes, have vastly improved options for high resolution chromosome studies in reptiles generally [Waters et al., 2009], and these kinds of high-resolution studies are needed in the genus *Sceloporus*.

Investigating the role of the sex chromosomes in promoting the evolution and development of sexual dimorphism is another fascinating question that is tractable in *Sceloporus*. The evolution of sex chromosomes in *Sceloporus* is dynamic (fig. 4), and the variation in morphology [Cox et al., 2003] and color [Wiens, 1999] between the sexes is high. Given that the sex chromosomes are often the only portion of the genome that differs between males and females, it will be interesting to determine the fate of the 'missing' XY system (e.g., those in which the X and Y may be identical in size and thus indistinguishable in conventionally stained preparations) in lineages of *Sceloporus* that express different degrees of sexual dimorphism. Detailed investigations of clades with missing XY systems may offer important insights into the relative roles and mechanisms by which autosomes and sex chromosomes foster sexual dimorphism [Mank, 2009]. For those species in which males are clearly the heterogametic sex, it is still unknown whether the Y chromosome is the dominant sex-determiner, or if gene products produced on the X chromosomes determine sex in a dosage sensitive manner [reviewed by Graves, 2008].

Continued investigations into *Sceloporus* phylogeny are necessary to obtain a robust framework for comparative evolutionary analyses. There are a number of unresolved issues pertaining to *Sceloporus* phylogeny that will benefit from additional analyses utilizing more exhaustive taxon sampling and more loci. For instance, what is the specific order of divergence events among the *graciosus, jalapae, magister, pyrocephalus,* and *gadoviae + maculosus* groups? The phylogenetic relationships among these species groups are particularly weak and require further analysis. Species limits in some groups are unclear as a result of processes including deep coalescence and/or gene flow [Marshall et al., 2006; Leaché, 2009] and understanding lineage divergence in these systems will require a detailed population genetics approach. In addition, the estimated divergence dates for *Sceloporus* presented here could be refined dramatically with the incor-

poration of additional calibrations on the phylogeny. Within *Sceloporus,* geological events associated with the formation of the Baja California Peninsula and the adjacent islands offer a rich source of potential calibrations for species divergence events [Leaché and Mulcahy, 2007]. Finally, future evolutionary studies aimed at detecting significant shifts in species diversification rates in *Sceloporus* will benefit from the development of methods that can evaluate the influence of multiple variables (e.g., autosomes, sex chromosomes, biogeography, and morphology) simultaneously.

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