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Does Karyotype Structure Co-Occurrence Patterns in a Chromosomally Diverse and Species-Rich Clade of Lizards (Genus *Sceloporus*)?

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ABSTRACT

Aim: We investigated the biogeographic consequences of chromosomal speciation, or structural changes in chromosome arrangement that lead to reproductive isolation, an intriguing speciation mechanism with implications for the phylogenetic and geographic distribution of species and chromosomal diversity. Location. North and Central America. Taxa. A species-rich and chromosomally diverse clade of lizards in the genus *Sceloporus* which are known to have coincident bursts of speciation and karyotype diversity, and coincident patterns of chromosomal change and branching order. Some workers have suggested that the tendency of communities of *Sceloporus* lizards to include species with different karyotypes is a signal of widespread chromosomal speciation, but given the high karyotypic diversity present in *Sceloporus*, this may be due to chance rather than the effects of karyotypic speciation.

Methods: We gathered karyotypic, morphological, and biogeographic data on this chromosomally-diverse clade in order to assess whether sympatry patterns of *Sceloporus* species are structured by karyotype. If karyotypic rearrangements contribute to the creation or maintenance of new species in *Sceloporus*, then sympatric sister taxa should be more karyotypically diverged than allopatric sister taxa, and allopatric taxa should accumulate differences more gradually. We investigate whether species pairs with similar karyotypes are less likely to overlap geographically than expected by chance, and test whether karyotypic and geographic overlap between species pairs is related to divergence time. We pay special attention to cases of overlap between sister species. We also investigate whether *Sceloporus* communities are karyotypically overdispersed by comparing observed geographic distributions of karyotypic and phylogenetic diversity against phylogenetically-informed modeled distributions.

Results: We find little evidence for geographic signatures of chromosomal speciation and suggest that, while chromosomal speciation may have contributed historically to the spatial distributions of *Sceloporus* species, any geographic signature of this mode of speciation has been lost at long (> 10 Ma) temporal and broad (continental) spatial scales.

Main Conclusions: The spatial signature of chromosomal speciation is temporally restricted and the influences of other factors may have greater effects on species distributions over long time scales in this group.

Isaac W. Krone and Erin P. Westeen contributed equally to this study.

1 | Introduction

Species boundaries are essential to the maintenance of biodiversity (Harrison and Larson 2014). If nascent species can freely interbreed, they lose their distinct traits and fail to maintain their ecological niches (Gilman and Behm 2011; Seehausen 2006). In a standard model of speciation, species boundaries are produced by the gradual accumulation of mutations that create genetic incompatibilities, decreasing the fitness of hybrids or preventing hybridization altogether (Presgraves 2010). But not all changes to the genome are small; chromosomal rearrangements scramble a genome at the structural level, producing a number of effects that may accelerate or even cause speciation (Faria and Navarro 2010).

At the most basic level, chromosomal speciation supposes that chromosomal rearrangements contribute to reproductive isolation (White 1978). The observation that many species differ in chromosomal rearrangements and that hybrids between parents with different chromosomal arrangements are often infertile suggests that this process can drive speciation. However, evidence that structural rearrangements of chromosomes are sufficient to cause reproductive isolation is limited (Coyne and Orr 2004). Rather, chromosome rearrangements may affect sets of genes that do cause reproductive isolation (Noor et al. 2001; Rieseberg 2001; Navarro and Barton 2003). Further, if novel rearrangements arise as heterozygotes, which can be semisterile, how do they rise in frequency (Sturtevant 1938; Muller 1940)? In small populations where drift can be stronger than selection, the possibility remains open; however, population sizes must be extremely small to satisfy this requirement (Walsh 1982) and the probability of fixation falls nearly exponentially with population size (Lande 1979).

To account for the fixation of underdominant rearrangements, most models of chromosomal speciation assume allopatry or other geographic isolation (Rieseberg 2001). Under a model of chromosomal speciation, sister taxa that have become secondarily sympatric should then be more karyotypically differentiated than allopatric sister taxa; chromosomal incompatibility stabilises the species boundary and allows for sympatry without hybridization (Kandul et al. 2007). One exception is the statispatric model, which supposes that in species with small populations, as chromosomal differences accumulate between populations, speciation will result from reduced offspring viability of heterozygotes (White 1978). Hybrids arise in the center of the range and displace the ancestral species (White 1978). The spatial distribution of the chromosomally novel daughter species should therefore be allopatric or narrowly sympatric with the ancestral species, and hybrid infertility can ensure species stability in the case of further sympatry.

Under any model of chromosomal speciation, it should produce a pattern of increased sympatry between chromosomally distinct species. Spiny lizards of the genus *Sceloporus* (family Phrynosomatidae) have been suggested to exhibit such a pattern (Leaché et al. 2016). *Sceloporus* are among North America's most diverse and ubiquitous reptiles; the more than 100 species differ dramatically in body size, range from Canada to Panama, with a center of diversity in the central Mexican plateau, and occur in almost every conceivable habitat in North and North Central America (Roll et al. 2017; Rivera et al. 2021; Uetz et al. 2025). In addition to their taxonomic, geographic and morphological diversity, *Sceloporus* lizards are karyotypically diverse, exhibiting derived karyotypes ranging from $2n = 22$ to $2n = 46$ that differ from all other Phrynosomatid lizards with the ancestral $2n = 34$ (Hall 2009). Previous work has suggested the role of chromosomal divergence in *Sceloporus* speciation (Lowe et al. 1967; Sites et al. 1992; Hall 2009; Leaché and Sites 2010) and showed a burst in diversification coinciding with the evolution of a chromosomally-diverse clade between 10 and 20 Mya (Leaché et al. 2016). Leaché et al. (2016) suggest that the geographic distributions of *Sceloporus* species reflect a history of chromosomal speciation, remarking that 'based on a cursory examination of the current geographic distributions of species in relation to their karyotypes, closely-related species of *Sceloporus* with the same karyotype formula are not typically found in sympatry'. If *Sceloporus* diversity has been shaped by chromosomal speciation, we might expect communities to be karyotypically richer than expected by chance, and species pairs, and especially sister species, to be more likely to co-occur if they do not share a karyotype.

Here, we test for these patterns of karyotypic and geographic overlap among 73 species in the karyotypically-diverse *Sceloporus* clade (Leaché et al. 2016). We use two major approaches. First, we compile a map of *Sceloporus* species and karyotypic richness at 0.5 degree resolution based on available range data. We then simulate karyotype evolution across species and compare the relationships between species richness and karyotype richness recovered in these models to that found in the real world. Second, we compare sympatry and karyotypic similarity between species pairs to determine whether species are more likely to coexist with species from different karyotype groups than with their own. Given the variation in body size between species in this genus and the fact that body size is a strong predictor of habitat ecology (Westeen et al. 2023, Westeen 2024), we repeat these tests within body size guilds of *Sceloporus* to investigate whether karyotypic differences promote coexistence of ecologically-similar and closely-related species. We also examine whether coexistence patterns differ among species pairs of differing ages and investigate geographic and karyotypic overlap between sister species pairs (following Castiglia 2014). Examining the geographic expectations of chromosomal speciation can help inform the extent to which this mechanism has played a role in the speciation process of *Sceloporus* lizards and provide a roadmap for understanding co-occurrence patterns in other karyotypically-diverse groups.

2 | Methods

2.1 | Data

Data and code produced for this study are available at doi.org/10.5281/zenodo.17437943. We gathered data on *Sceloporus* karyotypes from published resources (Hall 2009; Leaché and Sites 2010; summarised in Table S1). A time-calibrated phylogeny for *Sceloporus* was taken from (Leaché et al. 2016; Figure 1). We complemented this karyotype data with body size data as maximum snout-to-vent length (SVL),

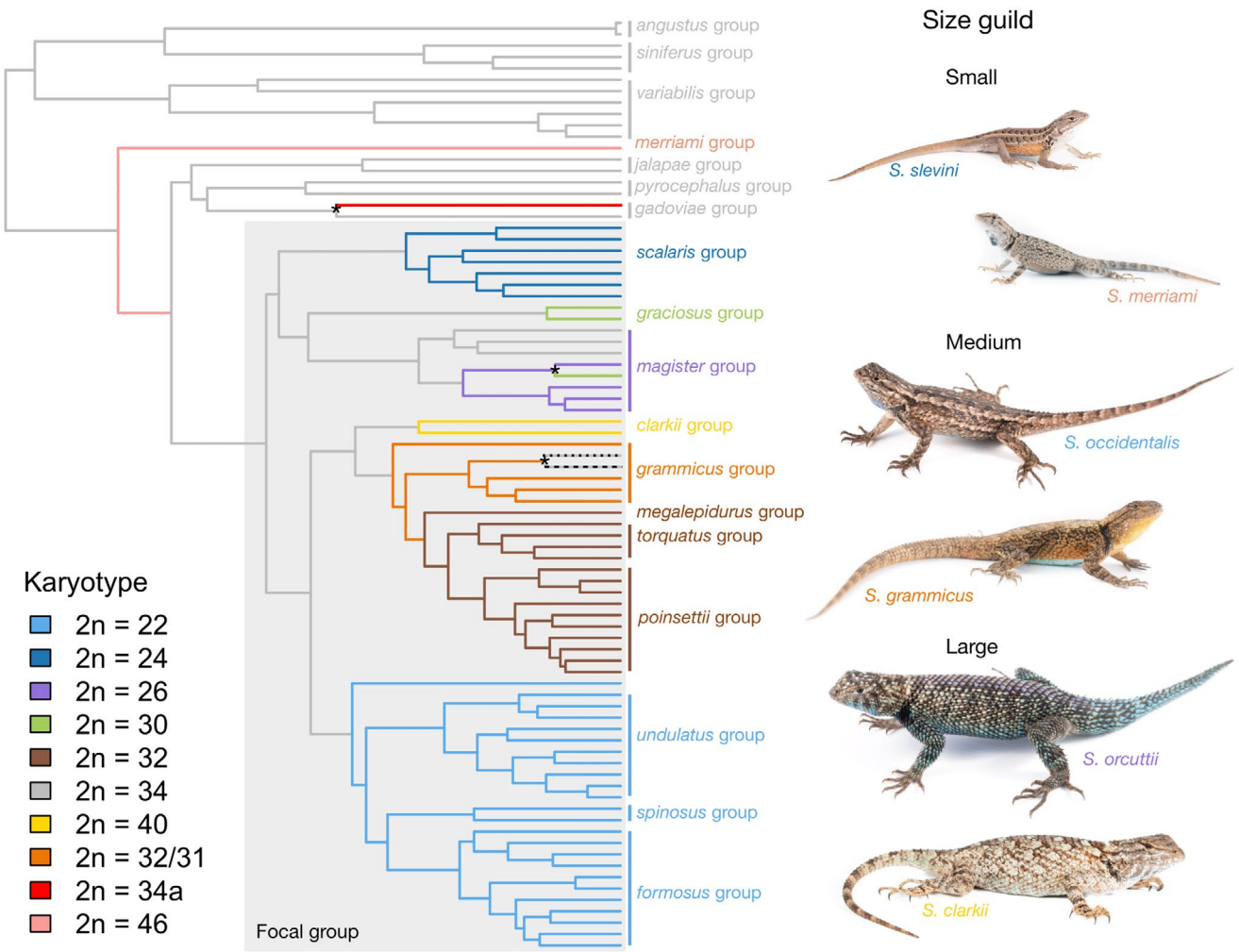

FIGURE 1 | *Sceloporus* phylogeny showing high levels of karyotypic diversity across the genus, modified from Leaché et al. (2016). Species groups are labelled with text and karyotype groups are indicated by colourised branches on the phylogeny. Branch colours reflect tip states and ancestral states were not inferred. Dashed lines represent two species in the *grammicus* group with distinct chromosome arrangements derived from $2n = 34$ (Hall 2009). *Sister species pairs with differing chromosome arrangements. Exemplar species for each size guild are shown, photos by Erin P. Westeen and José G. Martínez-Fonseca.

and endemicity data from Meiri (2018). To assess whether karyotypic differences facilitate coexistence between ecologically-similar species, we used body size as a proxy for habitat ecology and binned species into three size guilds: small species (SVL < 75 mm), medium-sized species (SVL 75–100 mm) and large species (SVL > 100 mm) based on natural breaks in the histogram of body sizes (Figure 1). Our 'small' size category corresponds with Rivera et al.'s 'small' and 'medium' size guilds, while our 'medium' and 'large' size guilds correspond to their 'large' size guild (Rivera et al. 2021). We downloaded species range data from the Global Assessment of Reptile Distributions (GARD) dataset, which provides range polygons based on occurrence records and expert opinion (Roll et al. 2017). As a second source of information about geographic overlap, we reconstructed a geographic overlap matrix from Rivera et al. (2021), who used occurrence data (rather than range polygons) to identify geographic overlap. Our operative dataset contains 73 *Sceloporus* species (hereafter 'focal group') in the chromosomally-diverse clade for which we have geographic data and are not insular endemics; 64 of these species are included in Leaché et al.'s phylogeny for *Sceloporus*, and this 64 species subset was used for all tests involving a phylogenetic component.

2.2 | Community-Level Comparisons

If karyotypic speciation is a driver of *Sceloporus* diversification, we should expect *Sceloporus* communities to have higher-than-expected karyotypic diversity so that karyotypically distinct but otherwise closely-related species can exist in sympatry instead of collapsing into a single species via hybridization. This should result in a relationship between species richness and karyotypic richness that is closer to 1:1 than expected by chance. To test this expectation, we used linear models to assess the relationships between species richness, karyotypic richness and phylogenetic relatedness across *Sceloporus* communities.

Both species richness and karyotypic richness are counts, and we used nearest-taxon index (NTI) as a measurement of phylogenetic community structure. NTI quantifies the degree of

terminal clustering within a phylogenetic community relative to possible communities drawn from the phylogeny (Webb et al. 2002). Higher NTI values indicate communities with relatively more terminal clustering (communities composed of more closely-related species). If closely-related *Sceloporus* with the same karyotype tend to exclude each other from communities, we expect to find a stronger relationship between karyotypic and species richness in communities with a high NTI. Since karyotype is an evolved trait, we expect closely related communities (high NTI) to have lower karyotypic richness absent any other structuring factors (i.e., the effects of karyotypic speciation). We measured NTI via the *NTI.p()* function from the iCAMP R package (Ning et al. 2020), modified slightly to run in R 4.4.1.

Using the GARD dataset, we built a matrix of *Sceloporus* species presence in geographic grid squares at 0.5° spatial resolution for all *Sceloporus* species in the GARD dataset. We used this matrix to build rasters of species richness, karyotype richness, difference in species and karyotype richness, and nearest taxon index for four subsets of species: (1) All focal group *Sceloporus* represented in the Leaché et al. (2016) tree ($n = 64$); (2) all small-body-sized *Sceloporus* found in subset 1 ($n = 15$); (3) all medium-body-sized *Sceloporus* found in subset 1 ($n = 30$) and (4) all large-body-sized *Sceloporus* found in subset 1 ($n = 19$).

2.3 | Alternative Karyotype Distributions

To understand whether the relationship between species richness and karyotypic richness is closer to 1:1 than expected by chance, we compared correlations between species richness and karyotype richness in our dataset to correlations based on simulated karyotype richness values. To produce simulated distributions of karyotypic richness, we fit three Mk models of discrete character evolution to karyotype—equal rates, symmetric and different rates—using our karyotype data and the Leaché et al. (2016) *Sceloporus* phylogeny. We compared these models via AIC scores and used the transition rate matrix (Q) from our best model to produce a continuous-time reversible Markov model for the evolution of karyotype, which we used to simulate 1000 stochastic character histories for the phylogeny. This created 1000 alternative histories of karyotype evolution that are influenced by phylogeny alone, and therefore 1000 alternative distributions of karyotypes across the 64 focal-group *Sceloporus* species found in the phylogeny. We used these 1000 alternative karyotype distributions to generate 1000 maps of alternative karyotype richness values for our *Sceloporus* geographic communities. Finally, we compared these alternative distributions to the observed distribution to assess if the relationship between species richness and karyotype richness is different from a random expectation.

2.4 | Model Constraints and Model Selection

With these data, we performed linear modelling to measure the relationship between species richness (predictor), NTI (predictor) and karyotype richness (response). Since our interest is in whether the species: karyotype relationship is close to 1:1, we compared the species richness coefficient between models based on observed karyotype richness and those based on alternative karyotype distributions. Since the species: karyotype relationship must be 1:1 in all *Sceloporus* communities containing only one species, we excluded these communities from our analysis.

We fit a set of 10 linear models to our 64-species subset, comparing them via Akaike Information Criterion (AIC). We first fit five non-spatial models to predict karyotypic richness based on species richness and NTI: a standard gaussian regression using only species richness as a predictor (karyotypic richness ~ species richness), a standard gaussian regression including NTI as a predictor (karyotypic richness ~ species richness + NTI), a negative binomial regression using the same NTI inclusive formula, a poisson regression using the same NTI inclusive formula (both binomial and poisson regressions were fit in an attempt to account for skew in the underlying species richness distributions; Figure S1), and finally, a gaussian regression using species richness, NTI, and the interaction term between those terms (karyotypic richness ~ species richness * NTI). All of these models were implemented using the R *glm()* function. We compared these models via AIC and selected the best model as our default non-spatial model.

We then measured Queen's distance Moran's I, a measure of spatial autocorrelation in which neighbours are defined as any grid squares that share a range edge or corner, on the residuals of the selected non-spatial model to determine whether the residual error was spatially organised. Using that selected non-spatial model as a basis, we compared the performance of five spatial regression models: a spatial error model, a spatial lag model, a spatial Durbin model, a spatial autoregressive combined model and a general nested model, all implemented using the 'spatialreg' R package (see Pebesma and Bivand 2023). Again, we compared AIC values between these models, following Kissling and Carl (2008), and chose the best-fit model as our chosen spatial model for further analyses.

2.5 | Observed and Alternative Histories

We measured the species richness:karyotype richness relationship of each of our four subsets of *Sceloporus* species—small, medium and large-bodied species, and all species—using both our observed karyotype richness data ('observed models') and our alternative karyotype richness data ('alternative models'). Three linear models were fit to each of these datasets: a simple model and our selected non-spatial and spatial models. The simple model was standard Gaussian regression of species richness on karyotypic richness (karyotype richness ~ species richness). For our simple model and selected non-spatial model, we repeated the tests for all 1000 simulated alternative karyotypic richness maps. For our selected spatial model, we repeated the tests on 100 alternative karyotypic richness maps. We generated p-values for the observed species richness coefficients by counting the number of alternative species richness coefficient values that were higher than the observed coefficient value, then dividing by the number of alternative values.

To account for spatial autocorrelation in species' ranges, we repeated this procedure while explicitly incorporating the spatial structure of species and karyotype richness using our preferred

spatial autoregressive model, shuffling karyotype groups 500 times.

2.6 | Species-Level Comparisons

For 2628 possible pairs of *Sceloporus* species in our 73 species dataset, we recorded (1) whether their geographic ranges (GARD data; Roll et al. 2017) intersect using the 'sf' function *st_intersects*, (2) whether they have the same karyotype, (3) whether they belong to the same size guild, and what size guild that is, (4) whether they were found to overlap geographically by Rivera et al. (2021), (5) whether both the GARD data and Rivera et al.'s data consider the species to overlap geographically, (6) whether that species pair contains sister species according to Leaché et al. (2016) and (7) the phylogenetic distance between the species.

We then performed Chi-squared tests of independence to determine whether karyotypic similarity (having the same karyotype) influenced the geographic overlap of species pairs. We tested for association between karyotype and GARD geographic overlap among the total species group, among only species included in the GARD and Rivera et al. datasets, and among subsets containing small, medium-sized and large species.

Phylogenetic relatedness renders species pairs non-independent, which can bias Chi-squared distributions away from the expected values. Therefore, we also tested for associations between karyotype and GARD overlap among species pairs based on the age of their most recent common ancestor for the set of 64 focal species found in Leaché et al. (2016) phylogeny. We performed an ANOVA relating overlap category (no overlap, GARD range overlap only, karyotype overlap only, and GARD range and karyotype overlap) versus the age of the most recent common ancestor of a species pair. Because we expect phylogenetic relatedness to influence whether species' traits are similar, we also used a Cochran–Mantel–Hanzel test to distinguish differences in observed versus expected Chi-squared distributions between four species-pair groups through time; those with a most recent common ancestor age of 0–5, 5–10, 10–15 and 15–20 Ma.

To account for any bias in geographic overlap in the GARD dataset, we also tested for an association between karyotype and Rivera et al.'s geographic overlap, and for the 260 species pairs found to overlap in both GARD and Rivera et al. datasets. We also performed a Chi-squared test of independence to determine whether size-guild similarity influenced geographic overlap.

2.7 | Sister Species Comparisons

The signature of chromosomal speciation should be strongest among closely related species, since these species are most likely to hybridise with each other and collapse species boundaries. To investigate the relationship between sympatry and karyotypic similarity at the smallest timescale available in our dataset, we compared the pattern of sympatry and karyotypic similarity among all sister species pairs in the phylogeny of Leaché et al. (2016). Chromosomal speciation should produce higher rates of sympatry between karyotypically distinct sister species than between karyotypically identical sister species (Castiglia 2014).

We also investigated whether the relatedness of sister species influences their probability of geographic overlap to understand whether closely-related *Sceloporus* species are unlikely to coexist, as would be expected if hybridization often erases species boundaries within the genus. To do so, we performed a Welch's *t*-test to compare the distributions of node heights (ages) of karyotypically distinct and identical and geographically distinct and overlapping species pairs, calculated from the phylogeny of Leaché et al. (2016).

2.8 | Occurrence Versus Range Map Data

We compared geographic overlap as measured by the GARD dataset and by Rivera et al.'s occurrence data for the set of 47 species shared among our focal group and the dataset of Rivera et al. (2021) to assess the degree of consistency between the two data types on co-occurrence patterns.

3 | Results

3.1 | Community-Level Comparisons

The equal rates model was our best-fit model of karyotypic evolution in *Sceloporus* ($AIC_{ER} = 113.82$, $AIC_{SYM} = 219.17$, $AIC_{ARD} = 336.8$; $\Delta AIC = 105.35$), with transition rates between all karyotypes of 0.0019. The results of this model were used to generate the 1000 simulated evolutionary histories for karyotype that served as alternative distributions for our linear models explaining karyotypic richness.

Species richness and karyotype richness are fairly well-matched within *Sceloporus* communities (Figure 2a–l), particularly within the small-bodied subset (Figure 2j). The areas of greatest difference occur in the Sierra Madre Oriental of Northeast Mexico and the Madrean Sky Islands in Arizona and New Mexico in the United States.

The geographic structure of the nearest taxon index (NTI) for *Sceloporus* communities is not well-matched with that of species or karyotype richness (Figure 2f). A large area of generally high NTI communities occurs in a fairly species-rich zone spread between Texas and New Mexico in the United States, and Chihuahua, Coahuila, Nuevo León and Tamaulipas in Mexico. Smaller patches of high NTI communities can be found in areas of lower species richness, often close to the periphery of the *Sceloporus* range, likely indicating independent invasions of *Sceloporus* lineages into these areas. These patches include the transition zone between Mojave and Great Basin deserts (California, Nevada, Arizona, United States), along the Baja peninsula, in Southern Guatemala and in Florida, United States.

The best-fit non-spatial model for our complete dataset was a Gaussian regression with the formula (karyotype richness ~ species richness * NTI) (Table S1). Species richness and NTI were positively correlated, but karyotype richness and NTI were negatively correlated (Table 1, Figure S2). We confirmed

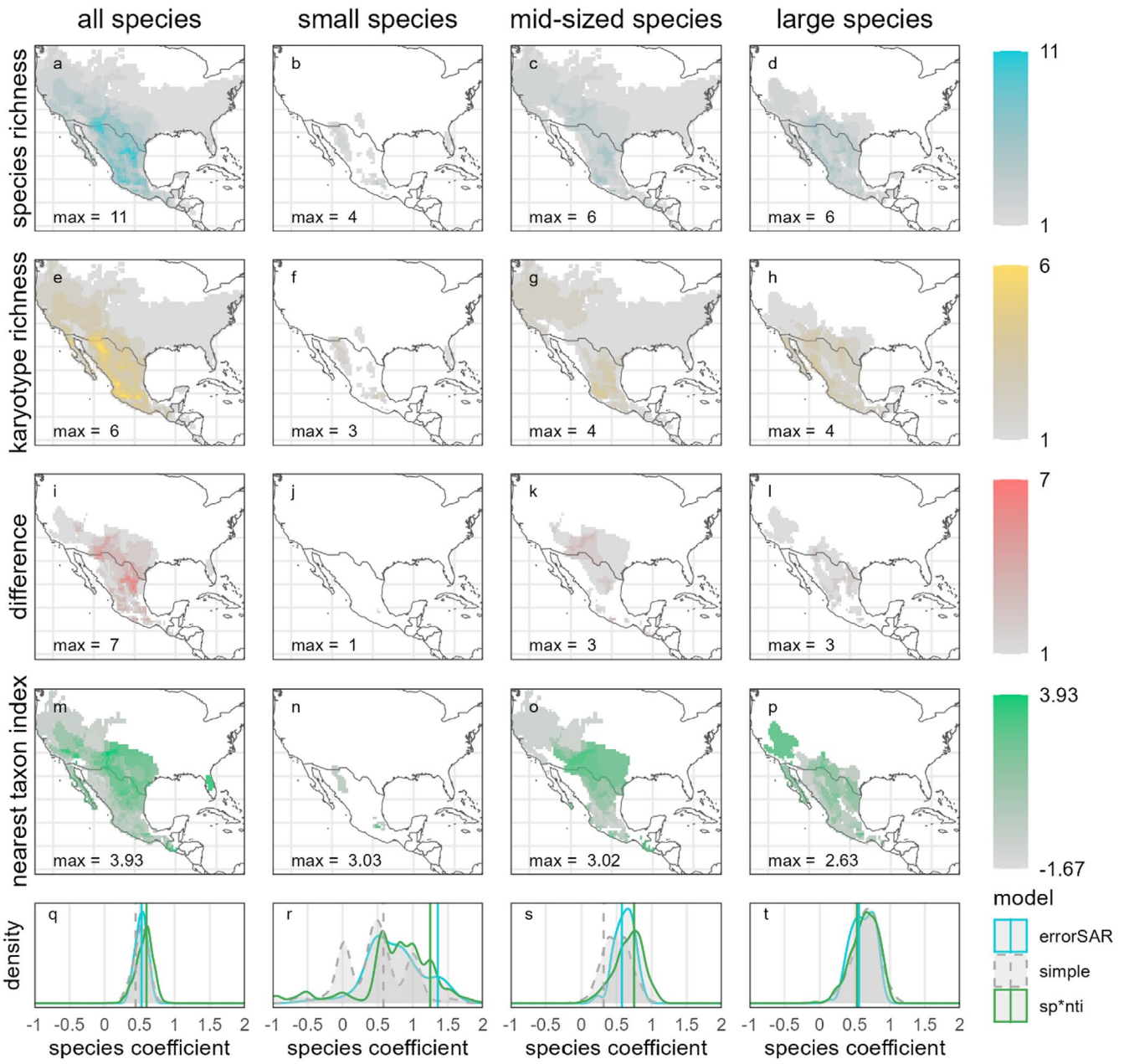

FIGURE 2 | Geography and correlations of *Sceloporus* species and karyotypic richness. The top four rows (a–p) map the species richness, karyotypic richness, difference in richness measures, and nearest-taxon index for 64 *Sceloporus* species in the chromosomally-diverse clade, as well as for small, mid-sized, and large size guilds within this group. The bottom-most row (q–t) shows the density of modelled coefficients for the species richness-karyotype richness relationship in simulations where community karyotypic richness was based on phylogenetically-informed alternative distributions of karyotypes, using simple (dashed grey lines), phylogenetically-informed (solid green lines), and phylogenetically and spatially-informed (solid blue lines) models. Vertical lines mark the modelled coefficient of the relationship between observed species and karyotype richness.

the presence of spatial autocorrelation in *Sceloporus* species richness (Moran's $I = 0.94$), and in the residuals of this preferred non-spatial model (Moran's $I = 0.76$), justifying the use of spatial models. Of our five spatial models, the spatial error model (errorSAR) performed best, so it was selected for further testing (Table S1).

In almost all of our models, we found that the observed relationship between species richness and karyotype richness in *Sceloporus* communities is not stronger than expected by chance (Figure 2q–t, Table 1). For the full set (64 species), as well as for the medium and large subsets, almost all modelled coefficient values for the species richness term fall between 0 and 1 in all three tested models, and the observed coefficient values were close to 0.5; quite far from a 1:1 relationship between species and karyotype. Within these datasets, our *p*-values comparing these observed coefficient values to those based on alternative karyotype distributions are also non-significant even before Holm–Bonferroni correction (Table 1).

Only among the small-bodied species do the results differ. In this smaller dataset (15 species), an overall lack of diversity of communities (9 unique communities, maximum of 4 species) limited the possible diversity of alternative karyotypic communities.

TABLE 1 | Results for regressions of species richness and nearest taxon index (NTI) against karyotype richness within *Sceloporus* communities.

Data		Regression results for observed data						
Subset	Model	McFadden's R^2	Intercept	Species richness coefficeint	NTI coefficient	sp:nti coefficient	Lambda	AIC
All	Simple	0.62	0.94	0.44	—	—	—	3633.11
All	sp*nti	0.88	0.72	0.60	−0.19	−0.07	—	1597.57
All	errorSAR	—	0.94	0.53	−0.15	−0.06	0.87	−33.29
Small	Simple	0.57	0.82	0.58	—	—	—	−46.29
Small	sp*nti	0.73	−0.61	1.25	−0.19	−0.05	—	−72.80
Small	errorSAR	—	−0.84	1.36	−0.22	−0.06	−0.28	−72.62
Medium	Simple	0.14	1.00	0.31	—	—	—	2526.57
Medium	sp*nti	0.93	0.20	0.75	0.05	−0.19	—	−962.85
Medium	errorSAR	—	0.60	0.57	−0.02	−0.14	0.88	−2068.90
Large	Simple	0.43	0.58	0.53	—	—	—	1382.20
Large	sp*nti	0.83	0.84	0.53	−0.24	−0.05	—	238.60
Large	errorSAR	—	0.76	0.56	−0.21	−0.06	0.87	−765.67

Data		Observed regressions versus regressions based on modelled karyotype distributions			
Subset	Model	p (species coefficient)	p (species coefficient, Holm–Bonferroni corrected)	p (NTI coefficient, Holm–Bonferroni corrected)	p (NTI-coefficient, Holm–Bonferroni corrected)
All	Simple	0.75	1.00	—	—
All	sp*nti	0.43	1.00	0.83	1.00
All	errorSAR	0.50	1.00	0.78	1.00
Small	Simple	0.31	1.00	—	—
Small	sp*nti	0.11	1.00	0.64	1.00
Small	errorSAR	0.04	0.48	0.63	1.00
Medium	Simple	0.82	1.00	—	—
Medium	sp*nti	0.40	1.00	0.53	1.00
Medium	errorSAR	0.62	1.00	0.67	1.00
Large	Simple	0.77	1.00	—	—
Large	sp*nti	0.73	1.00	0.54	1.00
Large	errorSAR	0.61	1.00	0.77	1.00

Our 1000 alternative karyotype distributions produced just 72 unique maps of karyotype richness for the small species subset, compared to 1000 in all other subsets. Model fit for regressions of the small subset using alternative karyotype distributions is quite poor; more than a quarter of the simple models (formula: kt ~ sp) had R^2 values below 0.1 ($n = 258$). Conversely, 133 of 1000 alternative karyotype distributions yielded models with R^2 values of exactly 1, indicating collinearity between species richness and karyotype richness (Figure S3). Analysis of the spatial error model with collinear richness datasets failed, as there was no residual error to explain. Therefore, the set of 100 alternative karyotype distributions tested in this dataset is slightly different from our other datasets, being the first 100 alternative karyotype distributions that were not colinear with the species richness data rather than the first 100 alternative karyotype distributions in the 1000-distribution set.

In all three models for the small-bodied subset, the distribution of coefficient values for the species term was quite broad (Figure 2r). However, the observed species richness coefficient in the spatial error model was significantly higher than expected by chance using a naïve threshold of $p < 0.05$ ($p = 0.04$), though not significantly higher when corrected for the number of models (Holm–Bonferroni corrected for $n = 12$: $p = 0.48$) (Table 1). In

TABLE 2 | Tests employed and results of analyses of geographic overlap between pairs of *Sceloporus* species.

Dataset	Formula	*n* species pairs	df	χ^2	*p*	Cramer's *V*
All focal species	Size guild ~ GARD range overlap	2628	1	0.839	0.36	0.018
All focal species	Karyotype ~ GARD range overlap	2628	1	0.107	0.744	0.005
Small focal species	Karyotype ~ GARD range overlap	120	1	*0.023*	*0.88*	*0.014*
Mid-sized focal species	Karyotype ~ GARD range overlap	630	1	0.85	0.356	0.037
Large focal species	Karyotype ~ GARD range overlap	210	1	0.129	0.72	0.025
Focal species in Rivera et al.	Karyotype ~ GARD range overlap	2080	1	0.221	0.638	0.014
Focal species in Rivera et al.	Karyotype ~ Rivera et al. range overlap	2080	1	2.135	0.144	0.044
Focal species in Rivera et al.	Karyotype ~ concurring range overlaps	2080	1	0	1	0

Note: Across all tests, we do not find a signature of geographic overlap associated with karyotype similarity. Within the small focal species subset, values from the Chi-squared approximation may be incorrect due to the small sample size.

our observed dataset, only 3 of 246 communities had different species richness and karyotype richness values (Figure 2j).

In nearly every model of observed data, NTI had a negative effect on karyotype richness; communities with more closely-related species tended to have fewer karyotype groups present. The coefficient values for NTI in our models of observed data were not significantly different from the NTI coefficient values in models using alternative karyotype distributions (Table 1).

3.2 | Species-Level Comparisons

We confirmed significant phylogenetic signal in karyotype (Blomberg's $K = 3.61$, *p*-value based on 10,000 randomizations < 0.001; Blomberg et al. 2003). Across our series of tests, geographic overlap between pairs of *Sceloporus* species was largely not associated with karyotypic similarity (Table 2). The results of our Chi-squared test of size guild and geographic overlap appear to concur with the results of (Rivera et al. 2021 and Westeen et al. 2025), who found that small *Sceloporus* species tend to co-occur with species of other size guilds, while large species (equivalent to our medium- and large-sized species) tend to co-occur with members of their own size guild. The low expected frequency of geographic overlaps within our small species subset made our estimates of the Chi-squared distribution for this group unreliable. These results still suggest no relationship between frequencies of geographic and karyotypic overlap among this subset of species.

We find one departure from expectations in our exploration of the relationship between phylogenetic age of species pairs and their overlap. As expected, MRCA age was strongly associated with karyotypic and geographic overlap, with all overlap categories significantly differing (Figure S4). The Cochran–Mantel–Haenszel test found that the common odds ratio among the four age groups of species pairs was not equal to one (Mantel–Haenszel $\chi^2 = 18.593$, $df = 1$, $p < 0.0001$), indicating that one or more of these species-pair groups departed from the common distribution. Only the cohort of species pairs with a most recent common ancestor between 15 and 20 Ma significantly departed from the Chi-squared expectation. The expected distribution included fewer geographically overlapping species (128 vs. 107.9 expected) but far more geographic and karyotypically overlapping species (7 vs. 27.1 expected) than we observed. In this cohort, the rate of geographic and karyotypic overlap was nearly a quarter of that expected, the opposite of the pattern of increased karyotypic overlap we would expect given the predictions of karyotypic speciation.

3.3 | Sister Species Comparisons

Three sister species pairs do not share a karyotype: *S. gadoviae* and *S. maculosus*, *S. zosteromus* and *S. lineatulus* and *S. anahuacus* and *S. palaciosi* (Figure 1). None of these species pairs occur in sympatry, according to both GARD data (Roll et al. 2017) and occurrence data (Rivera et al. 2021). Among the 23 sister species pairs which share a common karyotype, 12 have overlapping ranges. The small number of karyotypically distinct sister species pairs (3) means that it is impossible for a Chi-squared test to determine whether there is a relationship between shared karyotypes and range overlap. Nevertheless, the observed pattern is the opposite of that predicted; differing karyotypes do not facilitate coexistence of sister species.

The presence of geographic overlap is not related to the node age of sister species pairs. Welch's *t*-test ($t = -1.208$, $df = 23.729$, $p = 0.238$), and the node ages of the three karyotypically distinct sister species do not appear to be particularly young or particularly old.

3.4 | Occurrence Versus Range Map Data

Among 47 species of the chromosomally-diverse *Sceloporus* clade found in both datasets, GARD and Rivera et al. datasets find largely concurrent incidence of geographic overlap. For 1081 species pairs, the datasets concurred on the presence or absence of geographic overlap 993 times and disagreed 88 times, an 8% incidence of disagreement. Among these 88 disagreements, Rivera et al.'s dataset inferred overlap 32 times, while the

TABLE 3 | Summary of hypotheses, analyses used to test each hypothesis, data, and results from each set of tests regarding the biogeography of *Sceloporus* karyotype groups.

Test	Prediction (assuming chromosomal speciation)	Dataset	Result
Community comparisons	*Sceloporus* communities should have higher karyotypic richness than expected under a model where karyotypes are distributed to species using simulations of karyotype evolution	64 focal species in phylogeny	Negative
		15 small species	Insufficient data, but likely positive
		30 medium-sized species	Negative
		19 large species	Negative
Species pair comparisons	Sceloporus species that do not share a karyotype are more likely to live in sympatry than species that share a karyotype	73 focal species	Negative
		16 small species	Negative
		36 medium-sized species	Negative
		21 large species	Negative
Sister-species overlap	Sister species that do not share a karyotype are more likely to live in sympatry than sister species that share a karyotype	23 sister species pairs; 12 sympatric	Insufficient data, but likely negative
Overlap and age of species pairs	More recently diverged species pairs that overlap geographically are more likely to have different karyotypes	64 focal species in phylogeny	Negative

GARD dataset inferred geographic overlap 56 times, 21 of which were associated with *Sceloporus undulatus*. This likely stems from disagreement between the International Union for the Conservation of Nature (IUCN) range map and the GARD range map for *S. undulatus*. Rivera et al. used IUCN maps to bound occurrence points, and the IUCN map restricts the animal's range to the United States East of the Mississippi River (Leaché and Reeder 2002), while the GARD range map follows a much broader definition of *S. undulatus*, in which its range extends as far west as the highland range of Nevada in the United States and as far south as the city of Zacatecas in Zacatecas, Mexico.

4 | Discussion

Experimental (Delneri et al. 2003) and empirical (Ayala and Coluzzi 2005; Kandul et al. 2007; Castiglia 2014; Potter et al. 2017; Auvinet et al. 2020; De Vos et al. 2020; Palacios-Gimenez et al. 2025) evidence for chromosomal speciation provides grounds for authors to invoke it as a diversification mechanism in karyotypically diverse clades. Here, we focused on the geographic expectations under chromosomal speciation, namely that communities are karyotypically overdispersed, especially communities of closely-related species and sister species pairs. The karyotypic richness, species richness and frequency of co-occurrence of lizards in the genus *Sceloporus* makes this group an interesting test case; however, despite several approaches to the problem, we have found no clear signal that the biogeography of *Sceloporus* species is broadly structured by karyotypic similarity or dissimilarity (Table 3). At both species and community levels, karyotypic similarity appears to be largely unrelated to sympatry, even within size guilds, where ecological similarity might favour the collapse of karyotypically identical and closely-related species through increased interactions.

The possible exception to this lack of karyotypic structuring occurs among communities of small-bodied *Sceloporus* species. Our community analysis results suggest that the relationship between species richness and karyotype richness among small-bodied *Sceloporus* is particularly close (Figure 2j, Table 1). The most species- and karyotype-rich communities of small-bodied focal-group *Sceloporus* occur around Mexico City, home to several members of the chromosomally-diverse *grammicus* species group. The four species that occur within the most species-rich community in our small-bodied dataset are *S. aeneus* (scalaris group), *S. anahuacus* (grammicus-F6 group), *S. bicanthalis* (scalaris group) and *S. palaciosi* (grammicus-P1-HS group). The *grammicus* species group presents an interesting case of karyotype diversification within and between *Sceloporus* communities within the Valley of Mexico; while some believe that this could be evidence of rapid chromosomal speciation (Hall 2009), others cite intraspecific and intrapopulation polymorphism in karyotype as evidence that chromosome number differences alone may be insufficient to cause reproductive isolation (Hall 1980; Sites 1983). The limited variability in our dataset of small-bodied *Sceloporus* causes us to approach our statistical analyses with some caution, but we find the parallel between our coarse-scale result and the finer-scale analysis of *Sceloporus* karyotypic diversity compelling.

The interactions of sister-species pairs should produce the most informative data to test the hypothesis of karyotypic structuring,

and the lack of a large sample of karyotypically dissimilar sister-species pairs confounds statistical analysis of their propensity for geographic overlap. Our finding that none of the karyotypically dissimilar sister-species pairs overlap geographically does not support the claim that karyotypic dissimilarity promotes sympatry; if anything, it suggests the opposite.

In theory, rapid speciation due to chromosomal abnormalities could increase the number of sympatric congeners. By establishing reproductive barriers that would allow for rapid phenotypic divergence, these small, chromosomally novel populations could shift to a different niche due to competitive exclusion by the parent species. In practice, we find scant evidence of this process in *Sceloporus* lizards despite their remarkable karyotypic diversity, in contrast with other systems (Castiglia 2014). This does not discount the possibility that chromosomal speciation has occurred in these lizards, and an explicit analysis of speciation mode in relation to karyotype and other potential drivers would be a welcome follow-up (Tribble et al. 2025). But it does cast doubt on the idea that karyotype has been a major driver of *Sceloporus* community sorting.

At present, we find that the *Sceloporus* community structure is not karyotypically structured, but we suggest that this may reflect a mismatch in the scales of phenomena of speciation and community assembly, rather than a refutation of the action of karyotypic speciation within *Sceloporus*. We find the evidence of karyotypic speciation in *Sceloporus* compelling, but what little geographic evidence of the process we recover is consistent with a history of community assembly in which other factors are far more important. The modern distribution of *Sceloporus* species is a reflection of a deep evolutionary history. Leaché et al. (2016) recover a burst of speciation in *Sceloporus* from 20 to 25 Mya, corresponding with the evolution of several new karyotype groups. In the most recent 10 million years, *Sceloporus* speciation rates are estimated to have fallen, which could easily allow post-speciation range shifts to overprint any geographic signal of karyotypic speciation. At regional scales, most *Sceloporus* communities do harbor species from only a few different clades (Rivera et al. 2021, this study), suggesting that closely related species tend to remain in close geographic proximity or sympatry. But here is a hypothesis that bears further investigation: would a phylogeographic analysis of *Sceloporus* speciation and karyotype evolution support a chromosomal mechanism of speciation during the rapid radiation and accumulation of karyotypic diversity at 20–25 Mya (Leaché et al. 2016)? Future work leveraging comparative genomics, phylogeography, and explicit spatiotemporal models of community assemblage may illuminate the spatial and genomic context of diversification in this group.

A growing body of ecological and evolutionary research on *Sceloporus* lizards has identified multiple drivers of diversity in the genus, including biotic factors such as variation in body size (Rivera et al. 2021), parity mode (Lambert and Wiens 2013; Oufiero and Gartner 2014), and signal coloration (Romero-Diaz et al. 2019), and abiotic factors like habitat ecology (Westeen 2024, Westeen et al. 2025), elevational heterogeneity and mountain uplift (Bryson et al. 2012; Rivera et al. 2020, 2021) and bioclimatic change (Lawing et al. 2016). Along with karyotypic instability, these factors make *Sceloporus* lizards a particularly fruitful system in which to study drivers of diversity and their particular spatial and temporal signatures.

Author Contributions

I.W.K. and E.P.W. collected the data, ran the analyses, and wrote the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available at https://doi.org/10.5281/zenodo.17437943.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Selection of linear models for the relationship between karyotype richness, species richness and nearest taxon index (NTI) in *Sceloporus* communities. **Figure S1:** Quantile–quantile plots for distributions of species richness, karyotype richness, NTI values and residuals for the Gaussian regressions based on the 64-species dataset. **Figure S2:** Relationship between community species richness karyotype richness and NTI within the 64-species subset. **Figure S3:** Distribution of R^2 values for simple regressions of 1000 alternative karyotype richness maps versus species richness in the small-bodied *Sceloporus* subset ($n = 15$). **Figure S4:** Phylogenetic distance between species pairs compared to their geographic and karyotypic overlap.