Ecological, behavioral and phylogenetic influences on the evolution of dorsal color pattern in geckos

Short running title: Evolution of dorsal color pattern in geckos

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Abstract

The dorsal surfaces of many taxonomic groups often feature repetitive pattern elements consisting of stripes, spots or bands. Here we investigate how distinct categories of camouflage pattern work by relating them to ecological and behavioral traits in 439 species of gecko. We use phylogenetic comparative methods to test outstanding hypotheses based on camouflage theory and results in other taxa. We found that bands are associated with nocturnal activity, suggesting bands provide effective camouflage for motionless geckos resting in refugia during the day. A predicted association between stripes and diurnal activity was not supported, suggesting that stripes do not work via dazzle camouflage mechanisms in geckos. This, along with a lack of support for our prediction that plain patterning should be associated with open habitats, suggests that similar camouflage patterns do not work in consistent ways across taxa. We also found that plain and striped lineages frequently switched between using open or closed habitats, whereas spotted lineages rarely transitioned. This suggests that pattern categories differ in how specialized or generalized their camouflage is. This result has ramifications for theory on how camouflage compromises to background heterogeneity and how camouflage pattern might influence evolutionary trajectories.

Key-words: Animal Coloration, Animal Patterning, Camouflage, Compromise Camouflage, Functional morphology, Lizards

Introduction

The diversity of animal color patterns and their ease of observation have made this trait a popular subject for comparative investigations into the adaptive evolution of characters (Protas and Patel 2008; Cuthill et al. 2017; Endler and Mappes 2017). The adaptive causes of variation in camouflage coloration within species have been widely studied (reviewed in Protas and Patel 2008; Cuthill et al. 2017; Endler and Mappes 2017), however species-level variation in camouflage is not well understood. In vertebrates, large comparative studies on the drivers of camouflage diversity have only been conducted in a few groups (e.g. Ortolani 1999; Stoddard and Prum 2008; Wollenberg and Measey 2009; Allen et al. 2013; Halperin et al. 2017). There is a pressing need for macroevolutionary studies of animal coloration that aim to understand how different selection pressures influence the evolution of different color pattern phenotypes (Caro and Allen 2017). Here we use phylogenetic comparative approaches to investigate how the evolution of major categories of camouflage pattern such as stripes, spots and bands relate to differences in species' ecology and behavior to find our why there is diversity in camouflage pattern phenotype. Do key differences between species in habitat and activity time select for different camouflage patterns? This knowledge is essential for addressing outstanding questions in camouflage theory, such as whether some patterns make inherently better 'specialist' camouflage suited to one particular niche, or if some patterns provide 'generalist' camouflage suited to species that occupy a wide variety of niches (Ruxton et al. 2018).

In this work, we study these questions in geckos, a group of ca. 1744 species of Squamate reptiles. Geckos present an ideal opportunity to understand camouflage pattern diversity at macroevolutionary scales for two main reasons. First, we can confidently assume that dorsal pigmentation has been selected for a camouflage rather than signaling (e.g. warning coloration) function. The dorsal color gamut of is almost entirely restricted to earthy and neutral tones (Fig 1), with the exception of a few green arboreal taxa (e.g., genera *Naultinus* and *Phelsuma*), consistent with background matching camouflage. Experimental studies also support a camouflage function of gecko dorsal pigmentation (Vroonen et al. 2012; Ito et al. 2013; Fulgione et al. 2019), and camouflage is the primary function of dorsal pigmentation in other Squamate groups (Allen et al. 2013; Marshall et al. 2016). Except for the genus *Strophurus* (Nielsen et al. 2016), geckos are not known to possess significant secondary defenses which might support an aposematic strategy. Furthermore, coloration used for intraspecific signaling in geckos has only been identified on the head (Harmon and Gibson 2006, Nielsen et al. 2016), throat (Blouin-Demers et al. 2013), tail (Alonso et al. 2010, Nielsen et al. 2016), and through posture and movement for visual display (Marcellini 1977). Geckos are likely to benefit from camouflage through increased foraging success, but the primary selection pressure for camouflage is thought to be protection against visually-oriented predators such as birds-of-prey, snakes and mammalian carnivores (Ito et al.

2013). Being able to assume geckos' dorsal patterns function as camouflage facilitates investigation of how camouflage pattern diversity evolves and works by reducing noise that would be introduced when different selection pressures for non-camouflage functions lead to convergent patterns (Allen et al. 2013).

The second reason for investigating our research questions in geckos is their varied dorsal patterning, with species presenting spots, stripes and bands (Fig. 1), diverse ecology and behavior. About 30% of gecko species are mostly active during the day (diurnal) whereas other species are mostly active after sunset and in the first hours of the night (nocturnal) (Gamble et al. 2015). Geckos are globally distributed and occur in a variety of distinct habitats, including desert and sandy areas, trees and forests, urban environments, leaf litter, and rocky habitats. This species-level variation in camouflage pattern and hypothesized predictors of pattern variation makes the group ideal for comparative investigations. In geckos, the relationship between ecology and color pattern has only been investigated within single species or a few closely related species (Gübitz et al. 2000; Harmon and Gibson 2006; Saenko et al. 2013; Nielsen et al. 2016). At broader taxonomic scales, previous comparative studies of lizard coloration have excluded geckos on the basis of their generally nocturnal habits (Halperin et al. 2017; Murali and Kodandaramaiah 2017). We therefore do not yet know how pattern phenotype has responded to different selection regimes across geckos.

Although most gecko species are nocturnal, multiple lineages have reverted to diurnality (Gamble et al. 2015). This makes geckos an excellent group to test predicted relationships between activity time and camouflage pattern, which have not yet been thoroughly investigated. Predation on lizards by visually-oriented predators is expected to be higher during the day than at night (Poulin et al. 2001). To avoid predation, nocturnal geckos will use refugia and minimize movement during the day. We therefore predict that nocturnal geckos will utilize bands as camouflage because bands should be particularly effective background matching camouflage in typical refugia, such as rocky crevices and leaf litter, which have varying depth profiles that create high contrast shadows (Egan et al. 2016). Banded patterns should also provide better disruptive camouflage as more pattern elements intersect the outline. Disruptive camouflage through edge-intersecting patterns is an effective anti-predator strategy for motionless prey, but fails when prey move (Hall et al. 2013). We therefore predict that diurnally active geckos, who are more likely to be moving when seen by diurnal visually oriented predators, will utilize camouflage patterns thought to be effective during movement. Several lines of experimental evidence suggest that longitudinal stripes fulfil this criteria, providing effective dazzle camouflage that creates in predators an erroneous perception of the speed or trajectory of moving prey, facilitating escape (Scott-Samuel et al. 2011; Murali and Kodandaramaiah 2016). Since longitudinal stripes are more common on diurnal non-gekkotan squamates (Murali et al. 2018), here we test whether this is also the case in geckos. Support for these predictions would be good evidence that different categories of camouflage pattern have key mechanistic differences, and that these differences are maintained across different taxonomic groups.

Similar to variation in activity time, gecko habitat diversity also enables investigation of whether there are broad evolutionary relationships in how habitat structure relates to camouflage patterning, as has been observed for other reptile taxa (Allen et al. 2013; Halperin et al. 2017; Murali and Kodandaramaiah 2017), to determine whether there are any general 'rules' about how habitat influences camouflage pattern. To be as general possible we contrast open habitats, such as deserts, with closed habitats, such as forests. This has been shown to be a primary driver of camouflage diversity in other taxa. For example felids inhabiting open environments are more likely to be plain (Allen et al. 2011). In color changing animals, dorsal patterning emerges against darker backgrounds as would be found in closed habitats (Kang et al. 2016). The likely explanation for this is background matching against more visually homogenous backgrounds, whereas patterning evolves in more heterogeneous closed environments characterized by a variety of different surfaces and dappled illumination. We therefore predict plain patterning to be associated with open habitats in geckos and patterning with closed habitats.

We additionally investigate whether particular types of color patterns are selected as flexible solutions that are effective in a wide variety of habitat types, while other patterns are more specialized and only effective in particular habitats. Optimization of camouflage patterning against backgrounds that vary in appearance is an area of active theoretical and empirical development (Bond and Kamil 2006; Michalis et al. 2017), but it is rarely considered whether some patterns are inherently better 'general-purpose' coloration than others. In theory general purpose camouflage should evolve when animals utilize a wide variety of habitats with different background appearances (Ruxton et al. 2018). Generalist camouflage pattern phenotypes should be those that approximate the spatial pattern statistics across a variety of backgrounds. While some backgrounds such as vertical or horizontal vegetation feature oriented pattern elements, most gecko habitats are on average isotropic (e.g. sand, gravel, leaf litter), particularly when gecko movement is factored in. Therefore, we predict that geckos that inhabit multiple habitats will more likely be plain or feature isotropic spots, whereas more specialist geckos inhabiting a single habitat will more likely have anisotropic patterns (stripes or bands). This hypothesis is supported in snakes, where species with banded patterns tend to be habitat specialists (Allen et al. 2013). In this work, we therefore ask if this association is more general in squamate reptiles.

To address these questions, we carry out the first large-scale comparative analyses of 439 species (ca. 25% of all gecko species) belonging to all seven Gekkotan families to understand how habitat type, activity time, and habitat specialism influence the diversity of gecko dorsal color patterns. In addition to testing our main hypotheses, we conduct an exploratory analysis of all pattern types and eco-behavioral predictors to investigate further relationships that might inspire future studies.

Materials and Methods

Species selection and data collection

We developed a novel card sorting task to quantify gecko patterns to overcome practical issues associated with computational analysis of animal patterns for large comparative projects (Supporting Information 1). Beginning with all the species included in the most recent and complete phylogeny of geckos (Pyron et al. 2013), we built a gecko pattern dataset comprised of images available freely on the web. We followed a similar approach to Kelley et al. (2013) and checked a minimum of three images per species among those found through a Google Images search with the binomial name of the species (or synonym names) as the search term. Because geckos may have polymorphic coloration, including pattern polymorphisms existing between sexes and age classes (Johnston and Bouskila 2007; Booth 2008; Regalado 2012), two authors screened all images publicly available on the web for each species to select the one that showed the best view of the dorsal pattern for the most common pattern of adult males, in order to remove pattern variation due to ontogeny or sexual differences. To help ensure reliable species identification we preferentially selected images taken by one of the authors or hosted on well-known reliable herpetological websites, including The Reptile Database, ARKive and CalPhotos. A link to each image is available in Supporting Information 2 and images are also available on request. The images initially selected by two of us were then further checked by one of the other authors, who has the most extensive expertise of gecko identification and color patterning among the authors. Our final sample included an image of 439 species. Selected images were resized to the same length while keeping the original aspect ratio and printed in color on a 13 cm length cardstock, in which the image occupied the entire space of the card. No cropping of the image or image adjustment was carried out except for the length resizing, so the height of the printed images could be variable and the relative size of the gecko on each card could differ (Supporting Information 3).

In the card sorting task we allowed observers to freely sort patterns on a continuous pattern scale from transversely striped (“bands”) species at one end of the scale, through spotted species and then longitudinally striped species (“stripes”) with increased physical distance between images representing increased pattern difference. This quantification of finer similarities and differences between phenotypes enabled us to validate the categorization of patterns into distinct groups. Cards were sorted via a two-stage process. First two groups of seven observers each worked as independent groups, with the instruction to focus only on dorsal pattern (pattern occurring between the front and rear legs of each individual) and organize the images on each card into four discrete pattern categories (stripes, spots, bands and plain patterns, which corresponds to no pattern, Fig. 1) which were then piled on a table in a plain-band-spot-stripe order. In the few cases in which species had more than one type of pattern (e.g., spots and stripes), observers were asked to classify the card based on which pattern was visually more prominent. Image sorting from these two groups produced one pile with cards, for which both groups agreed on card classification in plain-band-spot-stripe order, with plain cards on the top and one pile consisting of cards that were assigned to different pattern types by the two groups (unassigned cards). We did not record which card belonged to which pattern for stage 1 observers, except for cards with plain patterns, which were not given to the stage two observers. The pile of band-spot-stripe and the one with discordant classification cards were combined into a single pile, with the unassigned cards from the stage 1 observers after the pile of cards in order band-spot-stripe. This single pile of cards was then given to seven additional observers in turn who each worked individually to arrange photos on a 1-dimensional scale. Stage 2 observers did not know how cards were ordered in the pile nor where one pattern category ended and another started according to stage 1 observers. The reason for the first stage was to make the task of arranging such a large number of images more tractable for stage 2 observes by having similar patterns already initially grouped together. The stage two observers however were free to disagree with the stage one observers' assessments and place them on the scale wherever they thought most appropriate. The seven stage two observers were instructed to place the cards along a line running down a hallway (44 m in length) in the band-spot-stripe order, with position within this constraint judged as they saw fit, without specific instruction on how each pattern should look for each category. Observers were allowed to overlap photos or leave space between photos to quantify perceptual difference (Fig. 2). This allowed observers significant freedom in determining what pattern attributes they considered perceptually most important, avoiding over-prescriptive instructions that produce classifications discordant with perceptually important variation.

When each observer had finished arranging the photos, they were then asked at what point on the scale the two pattern category boundaries lay (i.e. between bands and spots, and between spots and stripes), and these positions were recorded. The position of each image along the scale was then recorded using a tape measure and divided by the total length of the scale to give each image a continuous pattern score ranging from 0 to 1 for each observer. All observers were unfamiliar with the scientific aims of the study, except for one stage two observer, the author NM. Each image was assigned to a pattern category based on the majority categorical classification (dataset available as Supporting Information 4, *available after manuscript acceptance*). Inter-observer reliability for the continuous pattern scores was assessed using intra-class correlation coefficients, and inter-observer agreement for the categorical pattern judgments, was measured using Fleiss' kappa (Fleiss 1971). Additionally, to validate observers categorical classifications we used k-means clustering (n=3 clusters) on the continuous pattern scores and compared clusters using Cohen's kappa (Cohen 1960). To test the hypothesis that anisotropic patterns should be more common in habitat specialists we created a variable contrasting striped and banded geckos (0) with plain and spotted geckos (1).

Ecological and behavioral categories

To study the relationship between color pattern and the time at which a species is mostly active (night or day), we used the data from Gamble et al. (2015) to classify all the species in the dataset as nocturnal (0) or diurnal (1). We collected information on habitat type(s) using online species descriptions and published information about each species. Specifically, data on habitat occurrence for each species were obtained using the following strategy: 1) we used data from IUCN Red List and published scientific papers whenever possible; 2) when data were not available from the IUCN Red List or published papers, we used herpetology websites, such as the "Australian Reptile Online Database"; 3) if the information could not be found in either of these resources, we used field guides or general biodiversity websites such as the Encyclopedia of Life. We only considered habitats in which species were most commonly found and not occasional occurrences. We classified habitats as sand (sand dune/ desert), arid rock (rock outcrops or gravel plains in areas with sparse vegetation), shrub, forested rock (rock outcrops in forested areas), arboreal tree, and leaf litter, following the general habitat type categories used by the IUCN when available or using common category descriptors found on species descriptions on scientific papers or on the web. Each species was assigned a value of 0 (absent) or 1 (present) for each habitat category. Using these scores we also constructed a summary measure quantifying whether a species was more associated with open or closed habitats. Species were classified as occupying closed habitats (0) if they were present in more closed habitat types (leaf litter, forested rock, arboreal trees) than open habitat types (sand, arid rock); shrub habitats were considered intermediate and not included in this classification. Nine species were present in one open and one closed habitat. In these cases we further investigated the literature using the same sources to establish whether open or closed habits were preferred. All nine species had a preference for open habitats. Finally, species were classified as specialist or generalist based on whether they occupied a single habitat category only (e.g., arid rock uniquely) or more than one habitat category. We obtained habitat category and habitat generalism data for 369 species, of which 340 species had an open/closed score (the other 29 species were only found in shrubland).

Data analyses

We used the *Discrete* function in BayesTraits v3.0.1 (Meade and Pagel 2016), which implements Pagel’s (1994) method to test for correlated evolution between two binary traits. The traits analyzed were the pattern categories (spot vs. non-spot; stripe vs. non-stripe; band vs. non-band stripe; plain vs. non-plain; isotropic pattern vs. anisotropic pattern) and categorical eco-behavioral traits (open vs. closed; generalist vs. non-generalist; diurnal vs. nocturnal). We tested each combination of pattern category and eco-behavioral trait for a total of 15 analyses. The *Discrete* function tests for correlated evolution between two binary traits by comparing the strength of evidence for a dependent model, where the transition rate of one trait from 0 to 1 and/or 1 to 0 is dependent on the state of the other trait (e.g. that transitions from plain patterning to spots are more frequent when a lineage is nocturnal than diurnal), to that for an independent model, where the transition rates between the states of each trait are unrelated. The independent model has four parameters (0-1 and 1-0 for both traits) and the dependent model has a maximum of 8 parameters (0-1 and 1-0 for both traits when the state of the other trait is both 0 and 1). Support for the dependent model over the independent model was assessed by log Bayes Factors (BFs) greater than 2, which implies that the evolution of the two traits is linked, with the pattern of transition rates describing the strength and direction of the relationship (Pagel and Meade 2006).

Additionally, we used the *Multistate* function in BayesTraits to establish the rate of evolutionary transitions between the four pattern categories. To test for phylogenetic signal in the discrete traits, i.e. pattern categories and eco-behavioral traits, we used the phylo.d function in the R (R Core Team 2018) caper package v.1.0.1 (Orme et al. 2018) to calculate the D statistic (Fritz & Purvis, 2010) and test if each trait is conserved as expected under a Brownian model of trait evolution (D = 0) or have evolved randomly (D = 1). To run all comparative analyses, we used the squamate phylogeny of Pyron et al. (2013) to incorporate phylogenetic relationships between the species included in our dataset. The tree was scaled so branches had a mean length of 0.1 by multiplying branch lengths by 0.006514. This facilitates parameter estimation and interpretation in BayesTraits by avoiding all parameter values being very small (Meade and Pagel 2016). Full details on the MCMC procedure are provided in the Supporting Information 5.

As the BayesTraits *discrete* method can sometimes lead to erroneous interpretations when single evolutionary events have a dramatic effect on results (Maddison and FitzJohn 2014), we evaluated the robustnesss of our results using phylogenetic logistic regression (Ives and Garland Jr 2009) using the *phyloglm* function in the phylolm package in R (Tung Ho and Ané 2014; R Core Team 2018). This tests for linear relationships between pattern traits and the predictor variables, rather than complex trait relationships potentially identified by the BayesTraits method, but it provides a useful validation of any simple associations the *discrete* method identifies. To facilitate comparisons with the discrete results, we ran 3 separate models with habitat generalism, activity time, and habitat openness as individual predictors and the phylogeny as a random effect. P-values were assessed using the Benjamini–Hochberg procedure (Benjamini and Hochberg 1995) to control the false discovery rate.

Results

Gecko pattern classifications

All stage two observers produced an overall similar pattern gradient. Inter-observer reliability for the continuous pattern scores, measured by the intra-class correlation coefficient was 0.82, and inter-observer agreement for the categorical pattern judgments, measured by Fleiss' kappa was 0.74. Agreement between observers' majority- rule categorical pattern classifications and classification of continuous pattern scores into three categories using k-means clustering was also high (Cohen's kappa = 0.69). These scores indicate very good to excellent agreement between observers and between observers and k-means classification in categorical and continuous pattern judgments (Cicchetti 1994). Observers tended to place species with clear unbroken stripes or bands at either end of the scale, with dorsal patterns with broken stripes or bands, or patterns where both stripes or bands are present together with spots, being placed towards the central 'spot' portion of the scale. Forty (9.1%) species were classified by stage one observers as plain and were not given to stage two observers to arrange along a scale (see Methods), while stage two observers classified 125 species (28.5%) as having banded patterns, 229 species (52.2%) as having a spotted pattern, and 45 species (10.3%) as having a striped pattern.

Testing for the strength of phylogenetic signal in the categories of pattern traits using the D statistics showed that plain and striped patterning was highly conserved within lineages and, spots and bands were moderately phylogenetically conserved (plain: D = -0.035, P(D = 0) = 0.55, P(D = 1) = 0; stripes: D = 0.208, P(D = 0) = 0.27, P(D = 1) = 0; spots: D = 0.487, P(D = 0) = 0, P(D = 1) = 0; bands: D = 0.403, P(D = 0) = 0, P(D = 1) = 0.02). This confirms the necessity of using phylogenetically controlled analyses. The ancestral pattern at the root of the gecko phylogeny was estimated as striped (P= 0.16), spotted (P = 0.31) or banded (P=0.51), with plain pattern very unlikely (P = 0.02, Supporting Information 6).

Transition rates between longitudinal and transverse stripes, spots, and plain patterns

The multistate analysis of evolutionary transitions between plain, striped, spotted and banded geckos showed that of the 12 parameters, four were estimated as zero (i.e. they do not occur) in over 50% of posterior samples. These were plain to spots, plain to bands, stripes to plain and bands to plain. A second group of pattern transitions occurred at a relatively low rate (spots to stripes, spots to plain and bands to stripes, mean posterior estimate = 0.363), while the other transitions were generally grouped as occurring at a high rate (mean posterior estimate = 2.27, see Supporting Information 7 for a full summary of the top 10 models). The transitions among patterns are summarized in Fig. 3 and suggest that the pattern gradient we asked observers to classify images on has some evolutionary/developmental basis: transitions between pattern categories adjacent on the gradient are generally more frequent than transitions between separated pattern categories, except that stripes frequently become bands without transitioning through spots. Plain patterns frequently transition to stripes but stripes do not transition back to plain, rather spots revert to plain.

Gecko eco-behavioral traits

In our sample, 309 (70.5%) gecko species are classified as nocturnal and 129 (29.5%) species as diurnal. *Multistate* analysis of transitions from nocturnality to diurnality occurred at the same low rate as transitions from diurnality to nocturnality (0.207, equal rates in 98.5% of posterior models). The ancestral gecko was estimated to be nocturnal (P = 1).

169 (38.6%) species in our sample live mainly in ‘open’ habitats (‘sandy’ or ‘arid rocky’) whereas 171 (39 %) species live mainly in closed habitats (‘forested rock’, ‘trees’ or ‘leaf litter’). Habitat information could not be confirmed for 70 (15.9%) species, and 29 (6.6%) species live in shrubland, which was not classified as either open or closed. Transitions from closed to open habitats have occurred at a negligible rate (posterior mean rate = 0.02, 88.5% of posterior models had a zero rate), whereas transitions from open to closed have occurred more frequently (posterior mean rate = 1.189, above zero in 99.9% of posterior samples). Geckos were inferred to have evolved from an ancestor that lived in open habitats (P = 0.98).

Of the species with habitat data, 259 (70.2%) are ‘specialist’ species that were scored as only inhabiting one habitat type and 110 (29.8%) are ‘generalist’ species that inhabit more than one habitat type. Transitions from habitat specialism to generalism occurred at a lower rate (1.358, above zero in 99.9% of samples) than transitions from generalism to specialism (4.368, above zero in 100% of samples). At the root of the tree habitat generalism was the preferred state (P = 0.77).

Testing for phylogenetic signal in the eco-behavioral traits showed that activity time is highly conserved (D = -1.04, P(D = 0) = 1, P(D = 1) = 0), as is habitat openness (D = -0.663, P(D = 0) = 1, P(D = 1) = 0) and habitat generalism (D = 0.184, P(D = 0) = 0.178, P(D = 1) = 0.0), congruent with the low transition rates observed for these traits in the *multistate* analyses. The phylogenetic distribution of eco-behavioral traits and pattern categories is presented in Fig. 4.

Correlated evolution of gecko dorsal patterning and eco-behavioral traits

To investigate the evolution of the four main pattern categories, plain, stripes, spots and bands, we calculated BFs to establish evidence for dependent models of trait evolution, where the transition rates of one trait are dependent on the state of the other trait, over independent models, where the transition rates of pattern and eco-behavioral traits are not related. Additionally we tested our hypothesis that isotropic patterns (plain + spots) should be related to habitat generalism. Results (Table 1) showed strong support for the evolution of bands being associated with gecko activity time and indicate that the evolution of all pattern types (plain, stripes, spots and bands) is associated with whether the habitat is open or closed. There was no relationship between any of the pattern categories or pattern isoptropy and habitat generalism, or between plain, stripe or spot patterns and activity time. Repeat runs of models were all highly consistent and prior choice did not strongly influence posterior samples except for two uniform models that did not converge (Supporting Information 8-10). Unsupported dependent models were also non-significant in the phylogenetic logistic regression results (Table 1).

To investigate the nature of the dependent relationships indicated in Table 1, we examined the transition rate parameters of the models with highest posterior probability. These are illustrated in Fig. 5 and described in the next two sections, along with the complementary phylogenetic logistic regression results. The ten best supported models for each analysis are fully summarized in Supporting Information 11-15.

Activity time and bands

The model with highest posterior probability (10.85% of samples) was a two-rate model where nocturnal lineages with any other pattern type gain bands, and nocturnal lineages with bands lose bands at a high rate (mean posterior rate = 2.62) while other transitions occur at a lower rate (0.45, Fig. 5a.). Other models with high posterior support (Supporting Information 11) similarly found the high rate of gains and losses of bands when nocturnal but additionally set one or two parameters to zero in ways consistent with an association between bands and nocturnal activity, for example transitions to diurnal when banded (Supporting Information 11, model 2 6.53% of posterior sample), or losses of banding when diurnal to a high transition rate (Supporting Information 11, model 5, 4.52% of posterior samples), suggesting an association between bands and nocturnality. This was supported by the phylogenetic logistic regression which found a significant relationship between bands and nocturnal activity ($Z = -4.853$, $P < 0.001$). Overall this supports our hypothesis that bands are associated with nocturnal activity patterns and shows that lineages both gain and lose bands when they are nocturnal at a much faster rate than when they are diurnal.

Habitat openness and all pattern types

The evolution of plain and striped patterns is associated with whether a lineage utilizes open or closed habitats (Fig. 5b and c, Supporting Information 12 and 13). Transitions between open and closed habitats are frequent when plain (mean posterior rate = 6.61) or striped (6.12) compared to other pattern types. This suggests that plain and striped patterns do not strongly constrain habitat type. Furthermore, in both open and closed habitats species change from striped or plain patterns to other pattern types at a higher rate than other pattern types change to plain or striped patterns, confirming the results of the multistate analysis of pattern categories (Fig. 3).

In contrast while spots are gained and lost at the same moderate rate (2.40, Figure 5d) in both open and closed habitats, spotted lineages very infrequently switch between open and closed habitats or vice versa (0.05, Supporting Information 14). This suggests that spots can be an effective phenotype in both open and closed habitats, but that they prevent switches between open and closed habitat.

These results suggesting that the key difference between patterns is in their flexibility, is consistent with the phylogenetic logistic regression analysis, which showed no support for a simple association between habitat openness and plain (Z = -1.040. P = 0.299), striped (Z = -1.309, P = 0.191) or spotted (Z = -1.219, P = 0.223) patterns.

Geckos with bands transition from closed to open habitats at a higher rate (1.61, Supporting Information 15) than non-banded geckos (99% of posterior samples set this rate to zero). The rate banded geckos transition from open to closed habitats is also set to zero in 98% of samples. While bands evolve in closed habitats at a similar rate (1.50), overall this supports an association between banding and utilization of open habitats (Fig. 5e). This weak association was supported by the phylogenetic logistic regression, with a trend towards bands being associated with open habitats (Z = 1/8874, P = 0.059).

Discussion

Our results reveal at a macroevolutionary scale the major species-level ecological and behavioral drivers of gecko dorsal pattern variation. Broadly, we see that dorsal patterning is associated with activity time and habitat type. Overall, observed relationships are consistent with our assumption that gecko dorsal patterning functions as camouflage and provide insight into the direction and pace of dorsal pattern evolution, enabling evaluation of outstanding hypotheses in camouflage theory.

We inferred the ancestral gecko to be nocturnal, living in open habitats, but not specialized to one habitat type, and probably either spotted or banded. Our results confirm that the gecko radiation has transitioned to diurnality at least six times, produced hundreds of diurnal species, and subsequently reverted to a nocturnal lifestyle multiple times, making the group ideal for understanding how phenotypes adapt to this major change in lifestyle, especially in terms of visual changes (Roth et al. 2009; Gamble et al. 2015; Pinto et al. in press). In support of our prediction that bands should evolve as a camouflage defense against visual predators when the prey is likely to be seen motionless, we found that bands were associated with nocturnal activity in both the *discrete* and logistic regression analyses. Nocturnal geckos rest in concealed locations during the daytime (Aguilar and Cruz 2010). Banded patterns are likely to provide effective disruptive camouflage for nocturnal geckos that will be motionless when visually oriented predators are most active because the patterns intersect edges, breaking up the conspicuous outline (Cuthill et al. 2005; Hall et al. 2013).

In contrast, we did not observe direct support for the prediction that longitudinal stripes should be associated with diurnal activity. This relationship was found in a similar comparative analysis of non-gecko lizard dorsal patterning (Murali et al. 2018), though not in a study of snake patterning (Allen et al. 2013). The hypothesis that stripes should be associated with diurnal prey is based on the idea that they might work via a dazzle camouflage mechanism rather than background matching camouflage. Unlike cryptic strategies which ‘break’ as soon as prey move (Ioannou and Krause 2009), dazzle camouflage works while prey are in motion, with theory suggesting that some dorsal patterns such as stripes could make it harder for predators to accurately estimate the speed of trajectory of prey (Scott-Samuel et al. 2011). Although we did not directly test the relationship between stripes and gecko mobility, the lack of association between stripes and diurnality is inconsistent with this idea, as diurnal geckos are more likely to be seen while moving by visually oriented predators. Current experimental support for the theory of dazzle camouflage in animals is mixed and largely limited to human predators (Ruxton et al. 2018). In non-gecko lizards stripes tend to co-evolve with colorful tails after a lineage has evolved caudal autotomy (Murali et al. 2018), with the suggestion that dazzling body stripes support redirection of predator attacks towards detachable tails, a comparative association is also supported by experimental evidence (Murali and Kodandaramaiah 2017). In geckos caudal autotomy is common but only a few species have conspicuously colorful tails (e.g. *Sphaerodactylus townsendi*, Fig. 1G), and problematically for the ‘dazzle and deflect’ theory, none of these have striped bodies. Furthermore, these colorful tails may be differently colored between the two sexes and may be under sexual selection more than functioning in predator escape. However, given the rapid prey speeds at which dazzle effects have been observed in the lab, it is possible that geckos simply move too slowly for dazzle camouflage to be effective against their predators. Experimental results are also mixed on whether stripes would be more effective dazzle patterns than bands (Hughes et al. 2014; Hogan et al. 2016). Thus it may be that stripes simply represent an effective background matching or disruptive camouflage pattern in circumstances unrelated to activity time. For example, some striped species may be associated with visually linear microhabitats such as grass (e.g. some *Strophurus* and *Cryptactites*) or narrow branches (e.g. *Uroplatus lineatus*) where stripes may be more cryptic than blotches or bands. Further, it remains possible that striped patterns are associated with non-camouflage functions. For example, within *Podarcis hispanicus*, a species of lacertid lizard with polymorphic coloration, striped individuals are darker than banded morphs and give birth to lighter offspring, with the suggestion that stripes support the camouflage of an alternative ecotype that has a greater demand placed on pigmentation for thermoregulation (Ortega et al. 2015). This may also be the case at the species-level in geckos, though striped geckos do not obviously appear to be any lighter or darker on average than non-striped geckos.

Another important result of the analysis of how patterning relates to activity time was that bands were both gained and lost at a higher rate in nocturnal lineages than diurnal lineages. The same was not true for other pattern types. Species active at night are likely to be less exposed to visually oriented predators, so there may be relaxed selection on poorly camouflaged intermediate forms as populations shift to or from a banded phenotype that provides good camouflage to another well camouflaged pattern category. Why this might apply only to bands is unclear. Other studies of reptile dorsal coloration have found that banded patterns are often found on ‘sit-and-wait’ ambush predators (Allen et al. 2013). Ambush hunters aim to remain hidden from prey while motionless, utilizing microhabitats where they are especially cryptic. The majority of gecko species are considered primarily ambush hunters as opposed to active hunters, though species-level data was not available for us to include this variable in our analysis. It may be that banded nocturnal ambush hunting lineages are under selection to change to or from other pattern categories frequently as a consequence of being able to change (over evolutionary time) preferred ambush sites with different appearances, to an extent that is not possible for diurnal lineages.

In the analysis of how patterning relates to habitat openness, we observed simple correlated evolution between banded patterning and utilization of open habitats, whereas stripes, spots and plain patterns do not have any directional evolution with habitat openness. This general result was partly confirmed in the logistic regression analysis, with a trend towards bands being associated with open habitats, while other patterns showed no linear relationships. There was no support for our prediction that open habitats and plain patterns should be associated. While we did not make a prediction about how bands relate to habitat openness, bands may be adaptive background matching camouflage in open rocky environments where substrates are made up of surfaces at many different depths that produce high-contrast shadows. As well as background matching against areas of light and shade, banded geckos with ‘edge-enhanced’ appearances, where light patches are bordered by a lighter outline and dark patched by a darker outline, as in *Cyrtodactylus cf. intermedius* (Fig. 1D), increase the local internal edge contrast. By creating pictorial relief, this phenotype may improve disruptive camouflage in environments that contain surfaces at many different depths (Egan et al. 2016).

Examining transition rates in the analyses of how spotted, striped and plain pattern types associate with habitat openness found that lineages transitioned between open and closed habitats relatively frequently when they were plain or striped, but infrequently when they were spotted. This suggests that spots generally represent a more specialized camouflage, ill-suited to facilitating evolutionary transitions between habitats, whereas plain and striped patterns are a more flexible camouflage solution across habitat types. While this conclusion was not supported by any relationships between habitat generalism (the number of habitat categories occupied by a species) and pattern type, overall our results suggest that an important difference between major dorsal pattern categories are in terms of their flexibility; both stripes and plain patterns seem to facilitate transitions between open and closed habitats, while spots hinder them. Considerable work has aimed to understand the circumstances under which compromise camouflage that affords some protection against multiple backgrounds, or specialized camouflage against one background, might evolve, depending on the nature of the trade-off between the probability of detection against different backgrounds (Ruxton et al. 2018). To our knowledge this is the first evidence that broad dorsal pattern categories may be intrinsically better compromise camouflage. The mechanism underlying pattern flexibility would be interesting to explore. One possibility is that flexible pattern categories reflect image statistics across the range of backgrounds (Chiao et al. 2009; Fennell et al. 2018), for example stripes, but not other pattern types, may be a common feature in both open and closed habitats. Another is that in different circumstances pattern categories reflect utilization of alternative camouflage mechanisms that are (partially) independent from background matching, and so provide flexibility across backgrounds, for example that banded geckos utilize disruptive camouflage, and striped geckos dazzle camouflage. The lack of any association between patterning and habitat generalism, and isotropic patterns with generalism specifically, may be because our level of analysis and the way we scored generalism was insufficient for capturing how variable the appearance of backgrounds a species uses is. Alternatively geckos may be able to use positional behavior to make anisotropic patterns work as effective background matching camouflage against a range of backgrounds (Webster et al. 2008).

A consequence of analyzing such a large species sample was that we were unable to quantify and analyze within-species variation in camouflage. Some of the 1744 species of gecko show considerable variation between populations, age classes, and individuals of the same species (Regalado 2012; Kiskowski et al. 2019). However, it appears that color is more variable than patterning. Additionally, most pattern variation appears to be within pattern-category (e.g., spots of different sizes, density or arrangements), so incorporating intraspecific variation would be unlikely to significantly alter our findings. Additionally some gecko species can change color, with animals darkening over a period of minutes as they become cooler, enter lighter habitats, or become threatened (Vroonen et al. 2012; Ito et al. 2013). However, although geckos may change their body color darkening it or lightening it, the pattern does not change, it only becomes more or less visible because of the contrast with the rest of the body color. Therefore, the actual pattern is not influenced by the potential color change. Finally, our study does not include data on UV reflectance of the color pattern. UV reflectance data cannot be collected from the images used in this study. Furthermore, as this study focuses on color pattern used for camouflage and virtually all natural backgrounds are strongly UV-absorbing (foliage, earth, rocks, water, bark), we do not think ignoring potential UV reflectance is an issue for our analysis of camouflage patterning. Dorsal pattern polymorphism, sexual dichromatism and rapid color change have been widely investigated in lizards (Paemelaere et al. 2011; Medina et al. 2016) but very little in geckos (Johnston and Bouskila 2007), and together with further investigation on the role of UV reflectance of color and color pattern they can be key subject for future investigation.

The multistate analysis of evolutionary transitions between the pattern categories may reveal features of the developmental basis of pattern formation in geckos. Results show that all transitions between stripes, spots and bands are possible. Transitions from stripes to spots and bands, and between spots and bands are common while transition to stripes from bands and spots are less frequent. In contrast plain geckos transition first to stripes, and only fade to plain again from a spotted phenotype. This result mirrors the results of mathematical models of pattern formation and evo-devo results that demonstrate how, once a pigment pattern generating mechanism is operational, minor alterations to the developmental process are required to produce marked phenotypic differences (Murray and Myerscough 1991; Chang et al. 2009; Allen et al. 2013; Dhillon et al. 2017; Kiskowski et al. 2019).

In conclusion, comparing the results observed here with those from comparative analyses of camouflage patterning in other taxa suggest that there are few, if any, general rules spanning different groups about the relationship between camouflage pattern type and ecology and behavior. We did not find support for relationships that have been identified in other Squamate taxa, for example between stripes and diurnal activity (Murali et al. 2018) and whereas we predicted plain patterning would evolve in open habitats, partly on the basis of relationships observed in other taxa (Allen et al. 2011), we instead found evidence that banded patterning was associated with open habitats. This emphasizes that effective camouflage is often relatively specific to natural image statistics at the spatial scale predator-prey interactions in a given taxa take place at (Fennell et al. 2018). The potential for camouflage to be highly-specific to an individual, population or species' visual ecology, including the visual behavior of predators, is clearly reflected in the intricate camouflage of some gecko species, for example in *Ptychozoon kuhli* (Fig. 1E) the effect of bands on disrupting the outline is further enhanced through epidermal fringes and webbing. Perhaps the most interesting result of our study is in the role of pattern flexibility in camouflage pattern evolution. Plain and striped patterns were found to be more flexible than spotted patterns, facilitating transitions between open and closed habitats. This finding could have important implications for how camouflage patterning influences lineage evolution and diversification, so the result warrants further experimental work and comparative investigation in other groups.

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Table 1. Results showing support for dependent models of trait evolution compared to independent models in discrete analyses and a summary of phylogenetic logistic regression models. In the discrete analyses BF's > 10 indicate very strong evidence for the dependent model, 5-10 is strong evidence, > 2 is positive evidence and < 2 is weak evidence. Dependent models with positive evidence and significant regression models after Benjamini-Hochberg correction are indicated in bold.

		Marginal likelihood		**log Bayes Factor (BF)**	**logistic regression**		
		dependent	**independent**		**β**	**Z**	**P**
Activity time	plain	-185.10	-180.15	-9.89	0.70	1.13	0.26
	stripes	-220.15	-215.54	-9.21	0.83	1.50	0.13
	spots	-365.44	-365.66	0.43	0.64	2.12	0.03
	bands	**-318.77**	**-331.85**	**26.17**	**-2.03**	**-4.85**	**<0.001**
Habitat generalism	plain	-315.24	-312.70	-5.09	0.18	0.43	0.67
	stripes	-350.15	-349.19	-1.92	0.24	0.47	0.63
	spots	-460.68	-455.17	-11.02	-0.43	-1.86	0.06
	bands	-461.09	-454.91	-12.35	0.47	1.90	0.06
	isotropic	-494.80	-494.26	-1.08	-0.31	-1.37	0.17
Habitat openness	plain	**-227.78**	**-234.25**	**12.94**	-0.58	-1.04	0.30
	stripes	**-262.54**	**-271.30**	**17.52**	-0.91	-1.31	0.19
	spots	**-415.74**	**-419.57**	**7.66**	-0.32	-1.22	0.22
	bands	**-373.64**	**-379.78**	**12.27**	0.55	1.89	0.06

Figures:

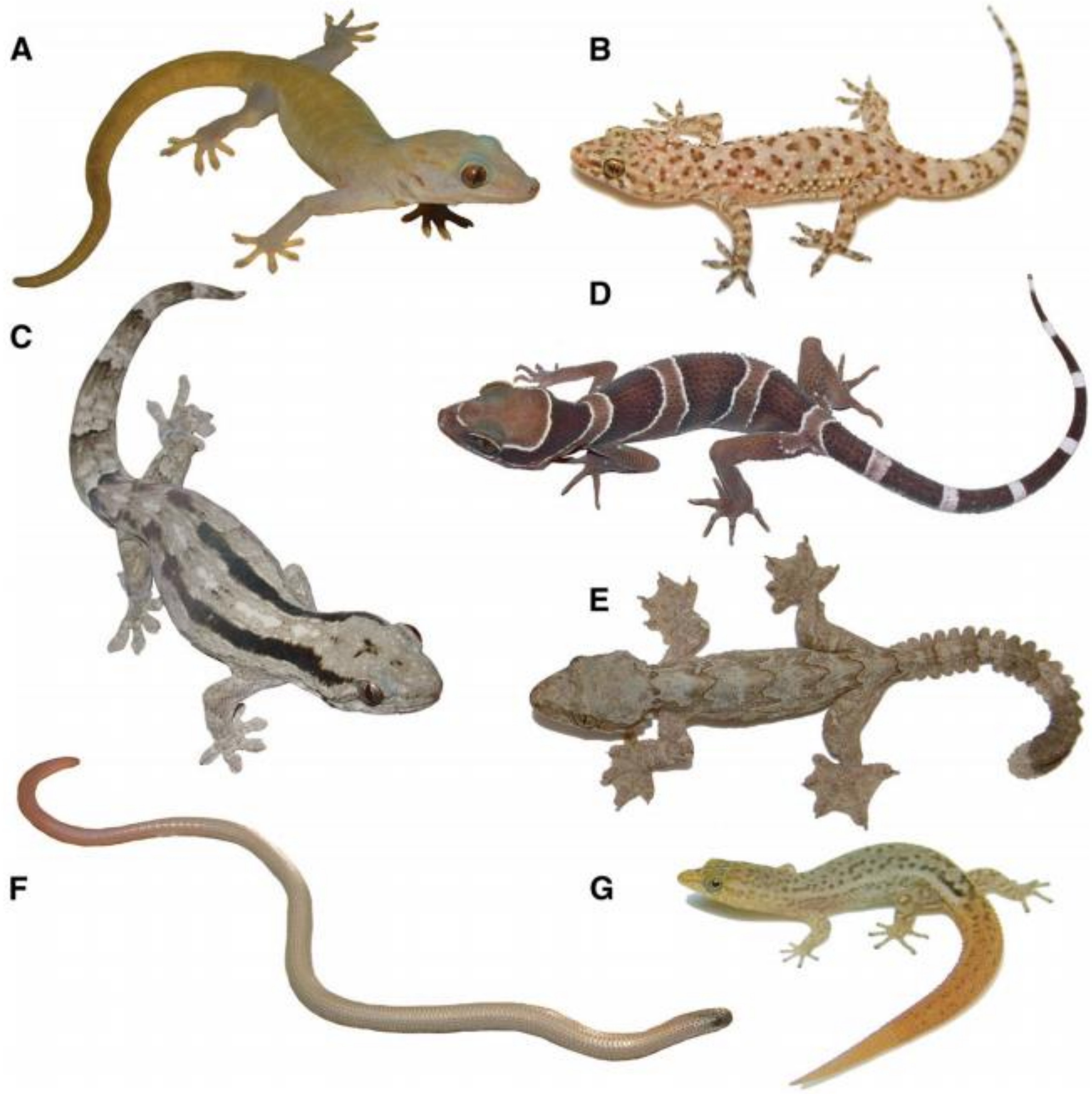

Figure 1. Dorsal pattern and color in several exemplar gecko species. A. *Gekko badenii*, plain pattern; B. *Hemidactylus turcicus*, spotted; C. *Homopholis arnoldi*, striped; D. *Cyrtodactylus cf. intermedius*, banded; E. *Ptychozoon kuhli*, a highly cryptic species with interdigital webbing and skin flaps that aid in concealment; F. *Aprasia parapulchella*, a limbless pygopodid with a pinkish tail; G. *Sphaerodactylus townsendi,* a diurnal gecko with an orange tail.

Figure 2. Printed cards organized along the 44m long hallway. Left image: Cards as organized by one of the stage two observers. Right image: The relative position of each card was obtained measuring its position with a tape ruler.

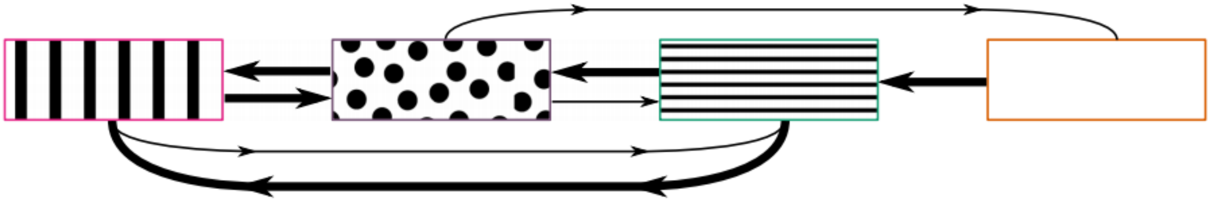

Figure 3. Transition rates between the four pattern categories. Thick lines denote a high transition rate and thin lines a low transition rate. From left to right, are bands, spots, stripes and plain pattern. Colors around each rectangle correspond to color coding used in Figures 4 and 5.

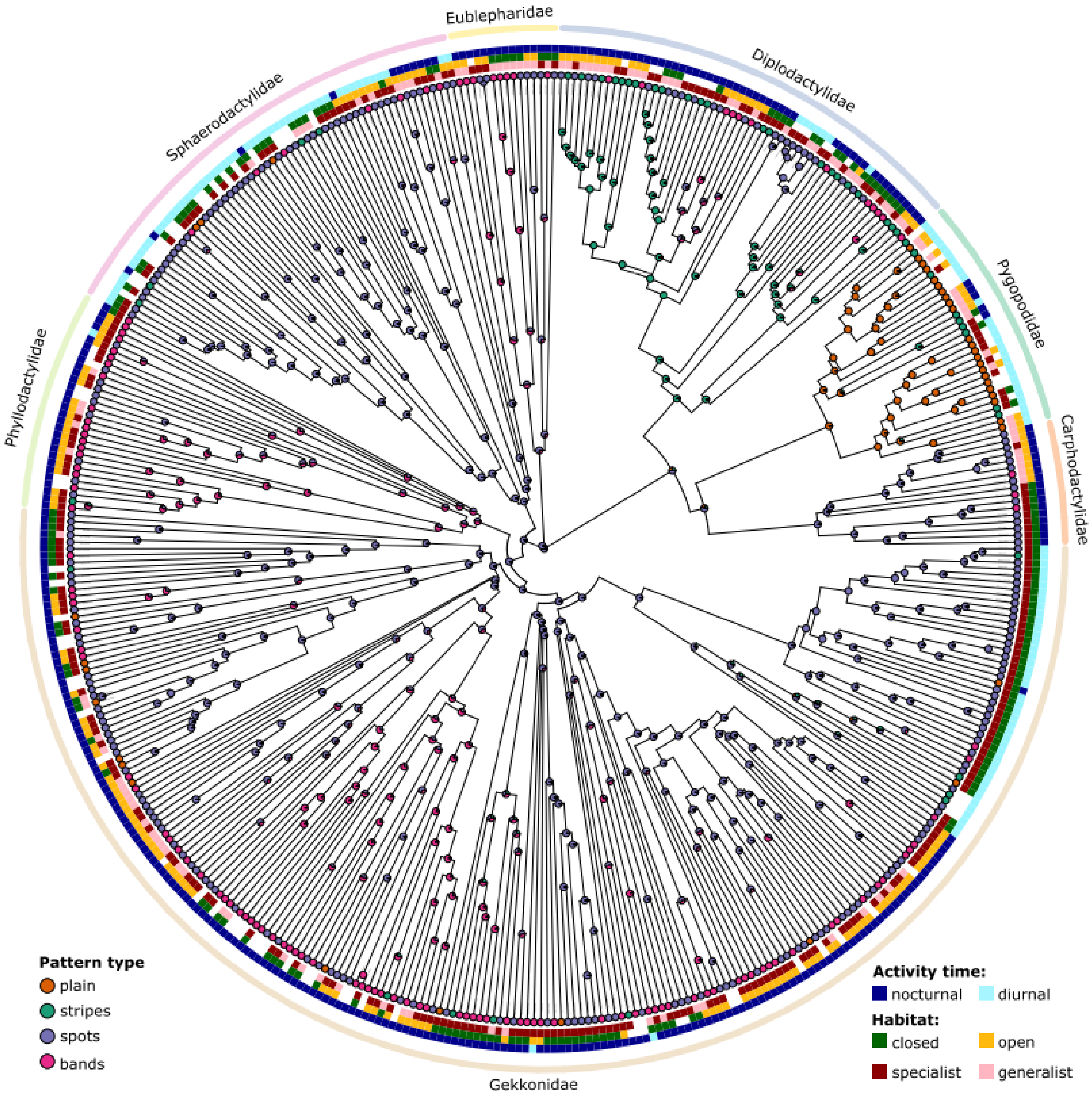

Figure 4. Phylogeny of the gecko species included in the present study with trait data, with gecko families indicated on the outer ring circle and identified by distinct colors. Circular symbols at the tips illustrate the four pattern categories, with orange corresponding to plain, green to stripes, purple to spots, and pink to bands. The pie charts at the nodes display the posterior probability of each pattern type, calculated using the *make.simmap* function in the phytools R package (Revell 2012) using the three-rate model and root state prior probabilities estimated by the *multistate* pattern evolution analysis (Fig. 3), simulating character histories 1000 times. The middle ring surrounding the tips shows data for habitat generalism, habitat openness and activity time scores.

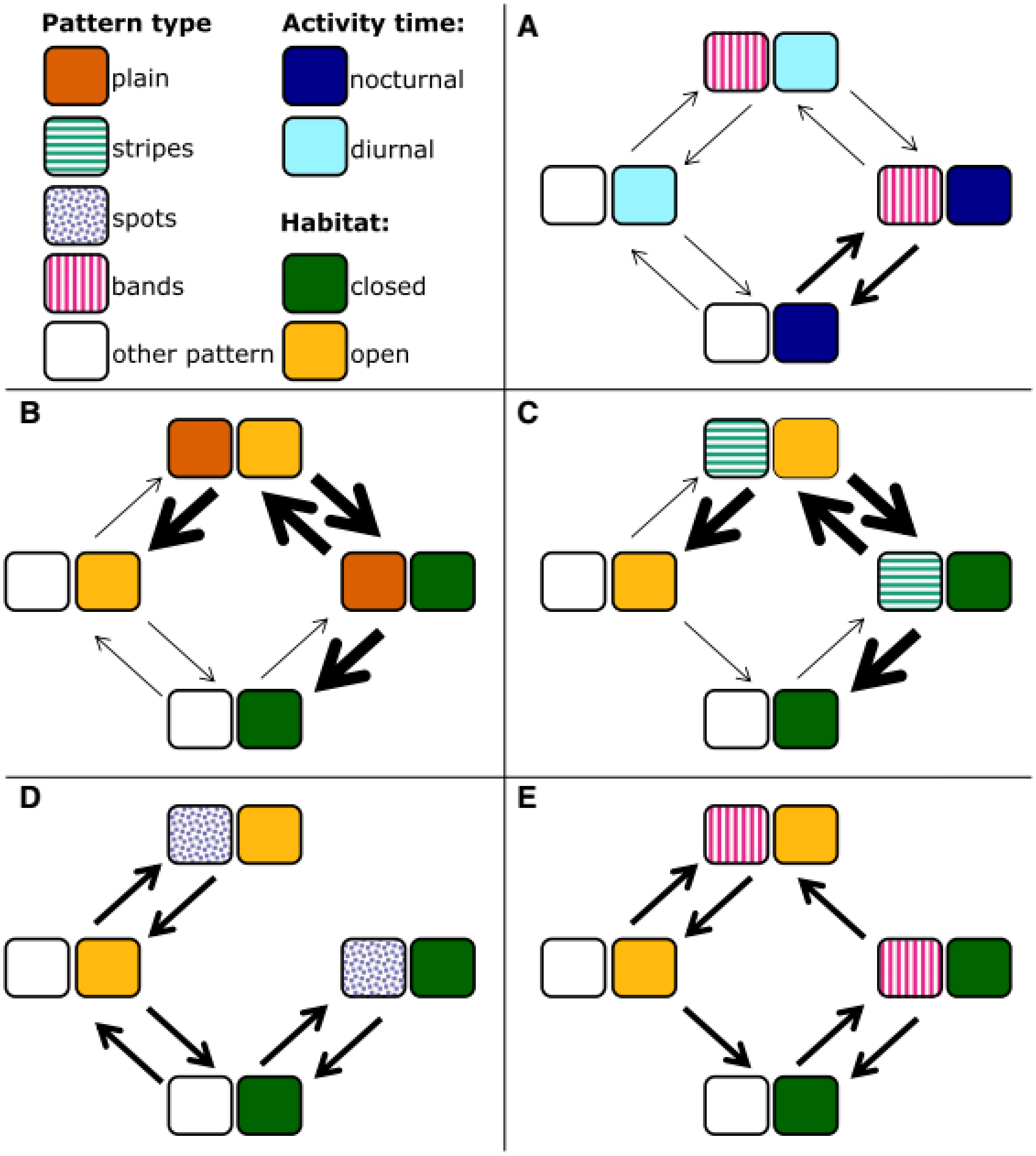

Figure 5. Estimated transition rates for the best supported dependent model of trait evolution for (a) activity time and bands, (b) plain pattern and habitat openness, (c) stripes and habitat openness, (d) spots and habitat openness and (e) bands and habitat openness. In the figure legend "other pattern" refers to all the other patterns excluding the one examined in each inset. The absence of an arrow indicates that the rate is zero, thin arrows indicate rates below 0.5, medium thickness arrows indicate rates between 0.5 and 3, and thick arrows indicate rates between 3 and 7.

Supporting Information:

Supporting Information 1: Additional explanation on card sorting task

Supporting Information 2: Links from where the images used in this study were downloaded. For each species used in this study, the link from where the image used was downloaded is indicated in the second column. If the link is currently not available anymore, a link of where the same image can currently be found is indicated in the third column. Species in bold are the ones for which the image is not available on the original link anymore or the link is not active anymore. Because for the majority of the images we do not hold copyright on any of the images used, as links may become inactive we will provide the exact images we used to interested readers upon request.

Supporting Information 3: Example of two printed cards used in this study. Length to width ratio of the image was respected and images were printed on a card at the same length (see Materials and Methods for additional information).

Supporting Information 4: Dataset, available after manuscript acceptance

Supporting Information 5: MCMC Analysis Procedure

Supporting Information 6: Summary of pattern probability at root of gecko phylogeny from multiple multistate transition rate models between the four pattern categories, using different priors on parameters.

Supporting Information 7: Summary of the 10 top models of transitions between the four pattern categories (plain = p; stripes = st; spots = sp, bands = b) as determined by the posterior probability (‘PP’). Results from ‘uniform prior run 1’ (Supporting Information 6). Transition direction denoted by ‘->’. Model summaries show groups of parameters set to the same rate in a sample (i.e. all ‘0’s have one rate and all ‘1’s have another rate. Parameters set to ‘Z’ have a zero transition rate. The mean, std and % zeros give the mean parameter rate, its standard deviation, and the percentage of samples that parameter was set to zero across all posterior samples.

Supporting Information 8: Summary of multiple runs of dependent and independent models testing correlated evolution of each pattern trait and activity time. For each pattern type we ran dependent and independent models nine times (three repetitions using uniform, exponential in gamma priors) to check that chains were stable and not strongly influenced by the choice of prior. As discussed in the main text, for some models with uniform priors, chains did not reach a stationary distribution, hitting upper limits on parameter bounds, so the result is not reliable. These are indicated by ‘*’s. Log BFs are reported both by comparing the number of visits to dependent and independent models in reversible-jump dependent models (model visit log BF) and by comparing log MLs for dependent and independent models (dep v. ind log BF), as described in Pagel & Meade (2006).

Supporting Information 9: Summary of multiple runs of dependent and independent models testing correlated evolution of each pattern trait and habitat generalism. See legend to Supporting Information 8 for further detail.

Supporting Information 10: Summary of multiple runs of dependent and independent models testing correlated evolution of each pattern trait and habitat openness. See legend to Supporting Information 8 for further detail.

Supporting Information 11: Summary of the 10 most frequently sampled posterior models of the dependent relationship between bands (bands = b, not bands = xb) and activity time (diurnal = d, nocturnal = n). Results from exponential prior run 2 (SI 8). Transition direction denoted by ‘->’. Model summaries show groups of parameters set to the same rate in a sample (i.e. all ‘0’s have one rate and all ‘1’s have another rate. Parameters set to ‘Z’ have a zero transition rate. The mean, std and % zeros give the mean parameter rate, its standard deviation, and the percentage of samples that parameter was set to zero across all posterior samples.

Supporting Information 12: Summary of the 10 most frequently sampled posterior models of the dependent relationship between plain (plain = p, not plain = xp) and habitat openness (open = o, closed = c). Results from exponential prior run 2 (SI 10). See SI 11 legend for further information.

Supporting Information 13: Summary of the 10 most frequently sampled posterior models of the dependent relationship between stripes (stripes = st, not stripes = xst) and habitat openness (open = o, closed = c). Results from exponential prior run 2 (SI 10). See SI 11 legend for further information.

Supporting Information 14: Summary of the 10 most frequently sampled posterior models of the dependent relationship between spots (spots = sp, not spots = xsp) and habitat openness (open = o, closed = c). Results from exponential prior run 3 (SI 10). See SI 11 legend for further information.

Supporting Information 15: Summary of the 10 most frequently sampled posterior models of the dependent relationship between bands (bands = b, not bands = xb) and habitat openness (open = o, closed = c). Results from exponential prior run 2 (SI 10). See SI 11 legend for further information.