Evolutionary shifts in habitat aridity predict evaporative water loss across squamate reptiles

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Aridity is an important determinant of species distributions, shaping both ecological and evolutionary diversity. Lizards and snakes are often abundant in deserts, suggesting a high potential for adaptation or acclimation to arid habitats. However, phylogenetic evidence indicates that squamate diversity in deserts may be more strongly tied to speciation within arid habitats than to convergent evolution following repeated colonization from mesic habitats. To assess the frequency of evolutionary transitions in habitat aridity while simultaneously testing for associated changes in water-balance physiology, we analyzed estimates of total evaporative water loss (EWL) for 120 squamate species inhabiting arid, semiarid, or mesic habitats. Phylogenetic reconstructions revealed that evolutionary transitions to and from semiarid habitats were much more common than those between arid and mesic extremes. Species from mesic habitats exhibited significantly higher EWL than those from arid habitats, while species from semiarid habitats had intermediate EWL. Phylogenetic comparative methods confirmed this association between habitat aridity and EWL despite phylogenetic signal in each. Thus, the historical colonization of arid habitats by squamates is repeatedly associated with adaptive changes in EWL. This physiological convergence, which may reflect both phenotypic plasticity and genetic adaptation, has likely contributed to the success of squamates in arid environments.

KEY WORDS: Climate, comparative methods, niche conservatism, phylogeny, physiological adaptation, water balance.

Because all terrestrial organisms face the risk of desiccation, aridity and precipitation are important components of the climactic niche for most terrestrial taxa (Quintero and Wiens 2013; Wiens et al. 2013). Consequently, aridity and related climactic factors can promote and constrain both ecological and evolutionary diversity (Crisp et al. 2004; Rabosky et al. 2007; Crisp et al. 2009). For example, although much of the higher-order plant and animal diversity in arid biomes has arisen from independent colonization by separate lineages, a substantial portion of the biodiversity at lower taxonomic levels often derives from radiations within lineages following ancestral adaptation to arid habitats (Chapple and Keogh 2004; Crisp et al. 2004; Byrne et al. 2008). This tendency for niche conservatism and "biome stasis" (Crisp et al. 2009) can give rise to phylogenetic conservatism in the traits that are putatively adapted to arid environments (Williams et al. 2004; Withers

et al. 2006; Oufiero et al. 2011). In a statistical sense, this means that tests for adaptation to arid environments must explicitly consider the phylogenetic history of the group in question (Williams 1996; Kleynhans and Terblanche 2009; Van Sant et al. 2012). In a functional sense, it also implies that the many morphological, behavioral, and physiological adaptations required for desert life may constrain the ecological and evolutionary dynamics of colonization, diversification, and assembly of communities (Chapple and Keogh 2004; Guerrero et al. 2013; Lanier et al. 2013). This has recently become a topic of considerable interest because these constraints, along with the evolutionary lag times they impose, may impede adaptation in response to climate change (Guerrero et al. 2013; Quintero and Wiens 2013).

Squamate reptiles (lizards and snakes) are often the most abundant vertebrates in extreme desert environments (Pianka

1967; Schall and Pianka 1978; Pough 1980; Morton and James 1988; Pianka 1989). Squamates that inhabit deserts exhibit a number of adaptations to desert environments, including changes in their thermal tolerance (Licht et al. 1966; Cullum 1997), locomotor mode (Secor et al. 1992; Pough et al. 1997), morphology and scalation (Cohen and Myres 1970; Carothers 1986; Oufiero et al. 2011), activity and thermoregulatory behavior (Muth 1977; Grant and Dunham 1988), and water homeostasis (Shoemaker and Nagy 1977; Davis and DeNardo 2010). Of particular interest, squamates from arid habitats exhibit lower rates of total evaporative water loss (hereafter EWL) than those from mesic habitats (Mautz 1982a, b), similar to patterns in birds (Williams 1996; Tieleman et al. 2003), mammals (Williams et al. 2004; Withers et al. 2006; Van Sant et al. 2012), and insects (Kleynhans and Terblanche 2009). EWL, the sum of respiratory and cutaneous water loss, is the primary avenue of water loss for many organisms (Shoemaker and Nagy 1977; Dawson 1982), and is therefore predicted to be a key determinant of fitness in arid environments.

Explicitly phylogenetic studies of squamates often indicate a tendency for greater diversification within, rather than across, arid and mesic habitats (Chapple and Keogh 2004; Rabosky et al. 2007; but see Fujita et al. 2010). This apparent niche conservatism, coupled with inferred lag times in adaptation to and diversification within arid habitats (Guerrero et al. 2013), suggests that (1) adaptation to arid environments may be a relatively gradual process that tends to constrain colonization and diversification, and (2) conclusions about the apparent adaptive match between EWL and habitat aridity in squamates (Mautz 1982a, b) may be premature in the absence of statistical tests that control for phylogenetic nonindependence (Dmi'El 2001). Analyzing evolutionary transitions in habitat aridity in concert with evolutionary shifts in the underlying mechanisms that facilitate adaptation is important because the plasticity or evolvability of physiological traits such as EWL should be an important determinant of the frequency of evolutionary transitions between arid and mesic environments.

To assess the relative frequency of evolutionary transitions in habitat aridity while simultaneously testing for associated changes in water-balance physiology, we combined a large dataset on habitat aridity for 860 lizard and snake species (about 9% of total squamate diversity) with published EWL estimates from 120 of these species and a recent molecular phylogeny of Squamata (Pyron et al. 2013). Due to incomplete taxon sampling, we could not estimate the actual number of transitions between habitats categorized as mesic, semiarid, and arid. Instead, we predicted that evolutionary transitions between arid and mesic extremes would be relatively less common than transitions to or from semiarid intermediates, assuming that our sampling is unbiased in its tendency to recover each type of transition in proportion to its actual frequency. Support for this prediction would be broadly consistent

with the idea that adaptation to habitat aridity is a gradual process, and that colonization and diversification may therefore be constrained by habitat aridity. Mechanistically, we predicted that rates of mass-specific EWL would be adaptively matched to habitat aridity, such that species from arid habitats would have lower EWL than those from semiarid and mesic habitats. Specifically, we predicted that phylogenetic analyses would reveal that evolutionary transitions in habitat aridity are consistently associated with adaptive changes in EWL.

Materials and Methods
COMPARATIVE DATASET

We generated a dataset of EWL estimates by augmenting Mautz's (1982b) compilation for lizards with more recent studies of EWL in both lizards and snakes (see Table S1, Supporting Information online). We included studies that measured EWL using three methods: (1) as the change in the mass of an animal in a chamber with airflow over a set time period at a particular temperature (e.g, Neilson 2002; Moen 2005); (2) as the change in mass of a chemical desiccant (anhydrous calcium sulfate or silicon dioxide) exposed to excurrent air from a chamber containing an animal at a particular temperature (e.g., Dawson and Templeton 1963; Cullum 1997); and (3) using gas analysis or freeze precipitation to measure water vapor and determine the difference in water content between incurrent and excurrent air from the animal chamber (e.g., Duvdevani and Borut 1974; Thompson and Withers 1997). For each study, we recorded mean rate of EWL (respiratory + cutaneous water loss, mg/h), mean body size (g), method of measurement (mass loss, chemical desiccant, water vapor analysis), and temperature during measurement. When multiple studies reported EWL of the same species, we preferentially included those with a measurement temperature closest to 30°C. When multiple studies measured EWL at the same preferred temperature for the same species, we preferentially included the study with the largest sample size. In one case when the same study measured two populations of the same species using the same sample size and temperature (Hillman and Gorman 1977), we used the average body mass and EWL across populations. Following Mautz (1982b), we assigned each species to one of three broad habitat types: (1) arid (e.g., desert); (2) semiarid (e.g., semi-desert, dry forest, and dry grasslands); and (3) mesic (e.g., moist forests and grasslands). We also included a fossorial category for completely subterranean squamates. This categorization revealed that fossorial species had exceptionally high EWL compared to all other habitat categories (Table 1). We excluded fossorial species from all subsequent analyses because (1) these species likely experience relatively moist subterranean conditions irrespective of the aridity of their surface environments (Shoemaker and Nagy 1977; Withers 1981), (2) relatively few species ($n = 9$) were included in

Table 1. Mean (± SEM) body mass (g), absolute EWL (mg/h), and mass-specific EWL (mg/g/h) for squamate species in each habitat type.

Habitat	n	Body mass (g)	EWL (mg/h)	EWL (mg/g/h)
Arid	43	16.41 ± 3.63	10.61 ± 1.65	0.93 ± 0.13
Semiarid	58	37.37 ± 17.49	35.19 ± 3.26	1.49 ± 0.28
Mesic	35	22.61 ± 6.66	35.98 ± 8.01	3.72 ± 0.70
Fossorial[*]	9	16.73 ± 11.74	90.77 ± 38.10	14.78 ± 6.21

Mean mass-specific EWL was calculated individually for each species because EWL does not scale isometrically with body mass, hence mass-specific EWL does not equal EWL divided by body mass. The two largest species of varanid lizard (both from semiarid habitats) are not included in this table because their body mass is an order of magnitude greater than most other species.

[*]Fossorial species were excluded from subsequent analyses due to their exceptionally high rates of EWL. See text for details.

this category, and (3) this category was phylogenetically biased, containing only scolecophidians and amphisbaenians. We also excluded two varanids (*Varanus panoptes* and *Varanus rosenbergi*) that were extreme outliers in body mass (1487 and 2411 g, respectively) and EWL (600 and 666 mg/h, respectively). Because we used this dataset to make inferences about the correlated evolution of EWL and habitat type, we only included species for which we had data on both EWL and habitat type.

We also compiled an expanded dataset on habitat type (without accompanying data on EWL of most species) for 860 species of squamate with known phylogenetic placement (Pyron et al. 2013). For this dataset, we included four large geographic regions with detailed field guides from which habitat aridity could be determined: Southeast Asia, North America, Central America, and Australia (see Table S2, Supporting Information online), which provided representative sampling of most major squamate clades. We assigned habitat type based on species descriptions, range maps, and climatic zones. We used this expanded dataset to test whether increased sampling across the phylogeny would change estimates of the relative frequencies of habitat transitions across squamates. We also compiled a dataset of 226 North American squamates for which geographic ranges of each species were examined to determine whether they border on ecoregions (following Omernik 1987; Omernik and Griffith 2014) that were classified as arid, semiarid, and mesic. To assess whether the geographic proximity of ecoregions might bias the probability of evolutionary transitions to and from various habitats (e.g., semiarid habitats may often bridge arid and mesic extremes), we counted the number and type of habitats (arid, semiarid, mesic) adjacent to the geographic range of each species (Conant and Collins 1998; Stebbins 2007), then used a Kruskal–Wallis test to ask whether species assigned to each habitat type differ in the median number of different habitat types bordering their geographic ranges.

NON-PHYLOGENETIC ANALYSES
We used a general linear model to test for a relationship between $\log_{10}$ EWL and habitat aridity with $\log_{10}$ body mass as a covariate. We initially also included measurement temperature (range 20–40°C, median 26.5°C) and method of measurement (see above), along with 2- and 3-way interactions of temperature with mass and EWL. Neither temperature nor method of measurement was statistically associated with EWL, so we removed these effects from subsequent analyses (see Table S3, Supporting Information online). The absence of a temperature effect is likely explained by the fact that we preferentially included estimates of EWL measured closest to 30°C when multiple estimates were available, which constrained the variation in temperature in our dataset. We used a reduced general linear model to test for a relationship between EWL and habitat aridity, also including body mass and the body mass x habitat interaction. We then tested for significant differences among least-squares means of EWL from this analysis using Tukey's HSD post hoc test. EWL and body mass were $\log_{10}$-transformed prior to analysis. These statistical analyses were conducted in JMP v. 9.0.1 (SAS Institute).

PHYLOGENETIC ANALYSES
We used the phylogeny of Pyron et al. (2013) to test for correlated evolutionary changes in EWL and habitat aridity. We first removed 17 species from the EWL dataset that were not represented in the phylogenetic tree, then pruned the phylogeny to remove species not included in our EWL dataset of 120 species. We used this pruned phylogeny to (1) test for phylogenetic signal in EWL and body mass, (2) reconstruct ancestral character states for habitat type and estimate transitions among habitat types, and (3) test whether evolutionary changes in habitat aridity predict changes in EWL. Phylogenetic analyses were conducted in the R environment (R Development Core Team 2013), and we manipulated the phylogeny using the R packages APE (Paradis et al. 2004), GEIGER (Harmon et al. 2008), and phytools (Revell 2012). We tested for phylogenetic signal using Blomberg's K (Blomberg et al. 2003), implemented in the R package Picante (Kembel et al. 2010). K is a ratio of the mean squared error of traits versus the mean squared error of traits calculated using the

variance/covariance structure of the phylogenetic tree, with significance (i.e., $K > 0$) calculated using simulation tests (Blomberg et al. 2003). If $K > 0$, related individuals tend to be phenotypically similar. If $K < 1$, phenotypic variance tends to be greater within clades than expected under evolution by Brownian motion, whereas if $K > 1$, phenotypic variance tends to be relatively greater among clades (Blomberg et al. 2003).

We reconstructed ancestral states for habitat aridity (treated as a discrete variable) using both stochastic character mapping and maximum likelihood with the ace function in the R package APE (Paradis et al. 2004) and the msim function (1000 simulations) in phytools (Revell 2012). These analyses were conducted for both the EWL dataset (120 species) and the expanded habitat dataset (860 species). Reconstructions were qualitatively similar using stochastic character mapping and maximum likelihood, so we present only the results from stochastic character mapping. We compared three potential models of transition probabilities between character states: equal rates (i.e., a single probability of transition from any character state to any other state), symmetrical rates (i.e., separate probabilities of transition between each habitat type, but with no difference in the directionality of transitions), and all rates different (i.e., separate probabilities of transition for each direction of transition between each habitat type). To evaluate whether transitions were more common between some habitat types than others, we compared these three models using corrected Akaike Information Criterion (AICc) with the fitDiscrete function in GEIGER (Harmon et al. 2008). Under each model, we used the posterior probability at each node to estimate the probability of each habitat type at each node, then estimated the number of transitions among habitat types in each dataset. For visualization, we rendered the phylogenetic tree ultrametric using penalized likelihood with the function chronos in the APE package (Paradis et al. 2004), and mapped posterior probability of each habitat to the nodes of this tree.

We tested for an evolutionary association between EWL and habitat aridity using a phylogenetic ANOVA (Garland et al. 1993) with mass-corrected residuals of EWL from the regression of $\log_{10}$ EWL on $\log_{10}$ body mass ($r^2 = 0.49$, $P < 0.0001$). This analysis was conducted using the aov.phylog function in the R package GEIGER (Harmon et al. 2008). This method calculates an F-statistic from a standard ANOVA, but estimates significance by simulating traits and calculating F-statistic distributions using the phylogeny (Garland et al. 1993). We also tested for differences among groups using post-hoc tests with P-values calculated from the same simulated distribution of F-statistics using the phylANOVA function in the R package phytools (Revell 2012). Similarly, we tested for a relationship between $\log_{10}$ EWL and ordinal habitat aridity (arid = 1, semiarid = 2, mesic = 3) with $\log_{10}$ body mass as a covariate. We implemented this phylogenetic generalized least squares (PGLS) analysis in the R package APE

(Paradis et al. 2004), using both Ornstein–Uhlenbeck and Brownian motion models of character evolution. For visualization, we also analyzed habitat as a discrete variable in a PGLS analysis to obtain the phylogenetically corrected EWL coefficients for each habitat type. Finally, we used phylogenetically independent contrasts to obtain contrasts of ordinal habitat aridity and EWL in the R package APE (Paradis et al. 2004).

Results

NONPHYLOGENETIC ANALYSES

EWL differed significantly among species from arid, semiarid, and mesic habitats (Habitat: $F_{2,130} = 11.10$, $P < 0.0001$) and scaled similarly with body mass in each habitat type (Mass: $F_{1,130} = 188.62$, $P < 0.0001$; Habitat x Mass: $F_{2,130} = 1.90$, $P = 0.1538$) (Fig 1A). EWL was significantly different for each pairwise comparison of habitat types (Tukey's HSD, all $P < 0.05$). Species from mesic habitats exhibited the highest EWL, those from arid habitats exhibited the lowest EWL, and those from semiarid habitats had intermediate rates of EWL (Fig. 1A). Geographically, the ranges of North American species from semiarid habitats border on a greater number of habitat types than do those of species from arid or mesic habitats (Kruskal-Wallis $P < 0.0001$; Fig. S1A). Whereas the geographic ranges of species from semiarid habitats frequently border both arid and mesic habitats (95% and 80% of semiarid species, respectively), the ranges of species from arid habitats rarely border on mesic habitats (11%), and those of species from mesic habitats rarely border on arid habitats (13%; Fig. S1B).

PHYLOGENETIC COMPARATIVE ANALYSES

We detected significant phylogenetic signal in body mass (Blomberg's $K = 0.278$, $P < 0.001$), EWL (Blomberg's $K = 0.326$, $P < 0.001$), and mass-corrected residuals of EWL (Blomberg's $K = 0.196$, $P = 0.008$). These values of K indicate that related species tend to be phenotypically similar ($K > 0$), but that variation is greater within clades, and lower among clades ($K < 1$), than expected under Brownian motion ($K = 1$) (Blomberg et al. 2003). Ancestral character-state reconstructions revealed some phylogenetic conservatism in habitat aridity, with the ancestral and deep nodes for some clades (e.g., dactyloid lizards) reconstructed as primarily mesic, while the ancestral and deep nodes for other groups (e.g., phrynosomatid lizards) were reconstructed as primarily arid (Fig. 2). However, character mapping also revealed evolutionary lability in habitat type, with multiple transitions between habitats detected even within primarily mesic- or arid-inhabiting clades (Fig. 2).

Models imposing a single probability of transition for all habitat types were significantly less likely than more parameterized models for both the EWL dataset (120 species) and the expanded habitat dataset (860 species, see Tables S5 and S6,
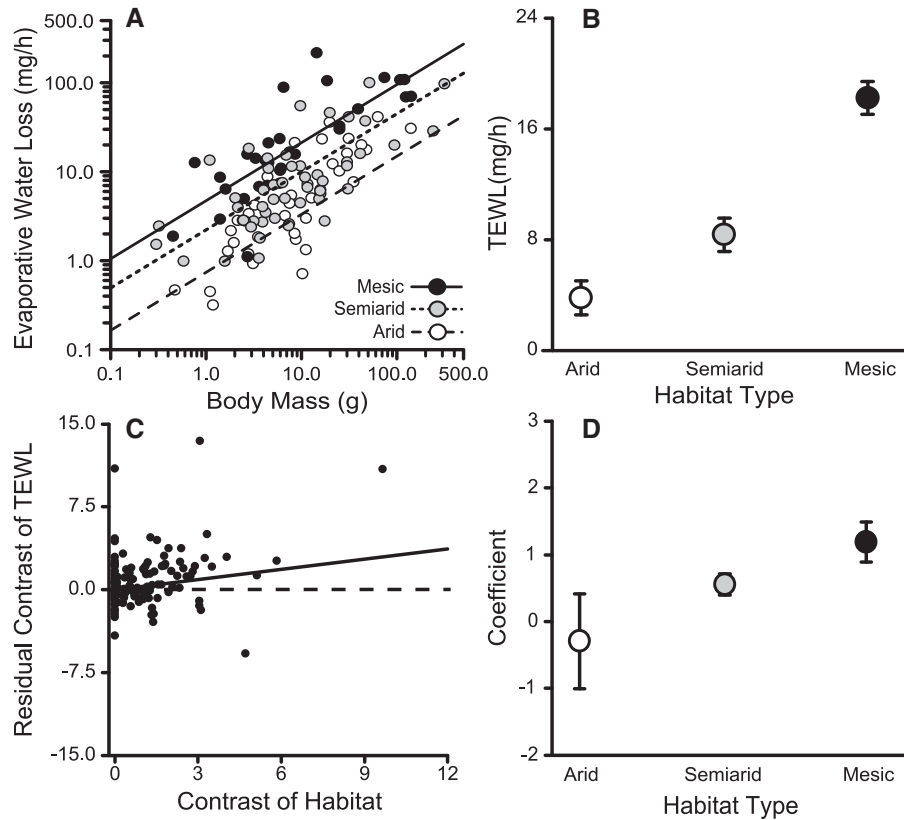
Figure 1. (A) EWL as a function of body mass and habitat aridity for 120 species of squamate reptile. (B) Least-squares mean EWL (± SEM) from a general linear regression with habitat type and body mass as predictor variables with interaction. (C) Relationship between standardized independent contrasts of EWL and habitat aridity (arid = 1, semiarid = 2, mesic = 3), such that large contrasts for habitat indicate evolutionary shifts toward increasingly mesic environments. Contrasts are "positivized" and the regression is forced through the origin (Garland et al. 1993). (D) Phylogenetically corrected coefficients of residual EWL (controlling for body mass) from a PGLS analysis with habitat as a discrete variable and assuming a Brownian motion model of character evolution (results are nearly identical using an Ornstein–Uhlenbeck model).

Supporting Information online). For the EWL dataset, symmetrical rates received the greatest support (Akaike weight = 0.56), whereas for the expanded habitat dataset, different rates of transition in each direction for each type of transition ("all rates different" model) received the greatest support (Akaike weight > 0.999, see Table S4, Supporting Information online; Fig. 3). In either case, likelihood-based comparisons rejected the hypothesis that the probability of transition was equal between all pairwise combinations of habitat (see Table S4, Supporting Information online). Examination of the number of transitions estimated under each of these models reveals that this result is driven by a high probability of transition between arid and semiarid habitats, an intermediate probability of transition between semiarid and mesic habitats, and a low (often zero) probability of transition between arid and mesic extremes (Fig. 3). Even when conservatively imposing equal rates of transition between all habitat types when reconstructing ancestral nodes (an assumption that is clearly not supported by the phylogenetic distribution of habitats, Fig. 2),

transitions involving semiarid intermediates were still estimated to be about 1.7 times more frequent than transitions between arid and mesic extremes.

Independent contrasts of mass-corrected residuals of EWL were significantly correlated with independent contrasts of habitat aridity ($r^2 = 0.31$, $P < 0.0001$, Fig. 1C). Habitat aridity and EWL were significantly correlated in PGLS analyses using Brownian motion (Habitat: $t = 5.17$, $P < 0.0001$; Mass: $t = 5.48$, $P < 0.0001$) and Ornstein–Uhlenbeck models of character evolution (Habitat: $t = 6.56$, $P < 0.0001$; Mass: $t = 11.17$, $P < 0.0001$; Fig 1C). EWL also varied significantly among habitat types when analyzed via phylogenetic ANOVA on mass-corrected residuals of EWL ($F_{2,117} = 12.252$, $P = 0.016$). EWL was significantly higher for species from mesic habitats compared to those from either arid or semiarid habitats (Bonferroni post hoc test from simulated data, $P > 0.005$; Fig. 1D), but species from semiarid and arid habitats did not differ in EWL (Bonferroni all-pairwise post hoc test from simulated data, $P > 0.99$).
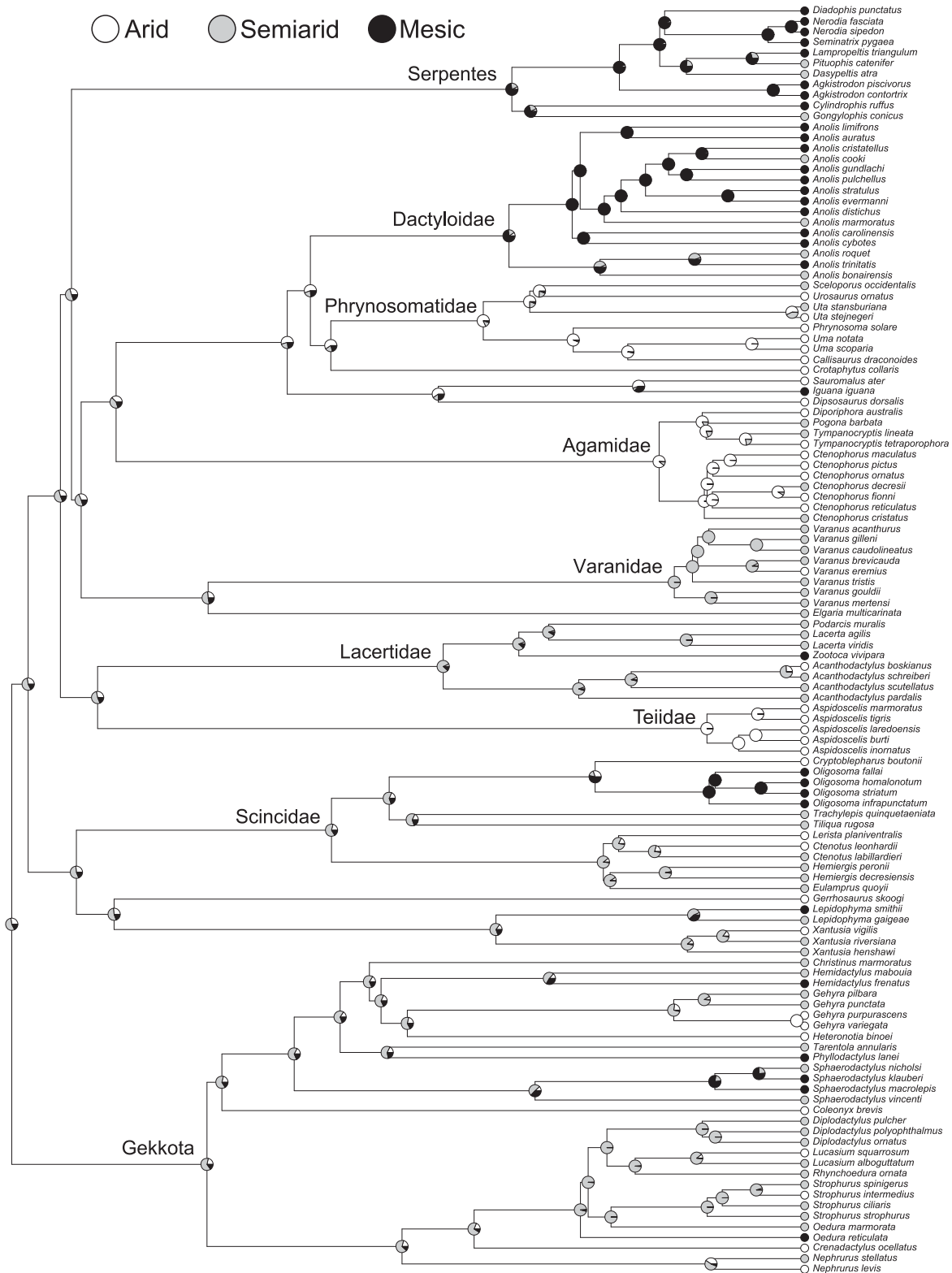
Figure 2. Distribution of habitat aridity for extant taxa and ancestrally reconstructed nodes across the pruned phylogeny from Pyron et al. (2013). The proportion of each color in the pie chart at each ancestral node is the posterior probability of each character state (habitat type) based on stochastic character mapping with a symmetrical-rates model of character transition.
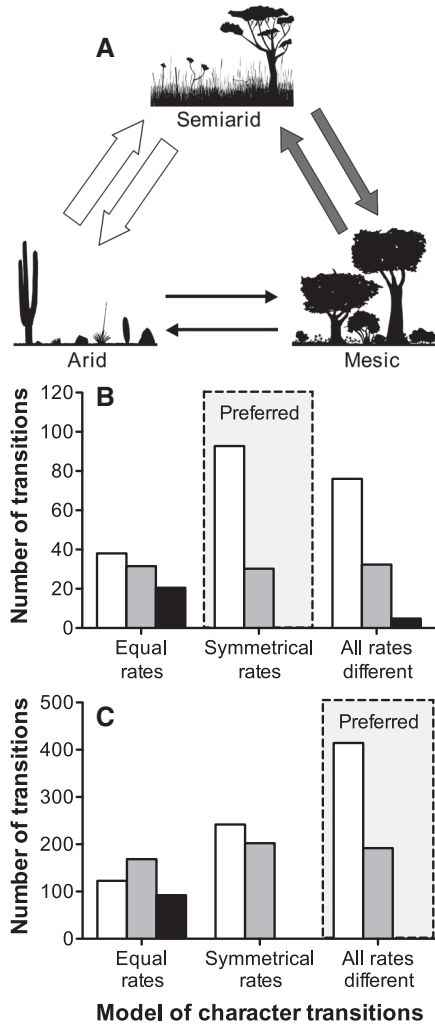
Figure 3. **(A) Illustration of the relative frequency of evolutionary transitions among habitat types for squamate reptiles. The width of the arrows between habitat types corresponds to the approximate frequency of transitions. (B) Estimated numbers of evolutionary transitions between habitat types within the EWL dataset (120 species) using stochastic character mapping with three different models of character transition. (C) Estimated numbers of evolutionary transitions between habitat types within the expanded dataset (860 species) using stochastic character mapping with three different models of character transition. For both datasets and using different models of character transition, evolutionary transitions involving semiarid intermediates are much more frequent than those between mesic and arid extremes. The colors of the arrows in A correspond to the colors of the bars in B and C.**

Discussion

Squamate reptiles exhibit an overall tendency toward phylogenetic conservatism in habitat type when classified according to arid, semiarid, and mesic habitats, such that character-state reconstructions tend to converge on one of these three habitat

types for deep nodes in many clades (Fig. 2). In other words, related species tend to share similar habitats, an evolutionary pattern often referred to as "phylogenetic niche conservatism" (Wiens et al. 2013) or "biome stasis" (Crisp et al. 2009). This pattern should be viewed as a broad generalization based on the relative frequency (rather than the actual number) of habitat transitions, with the important caveat that our taxon sampling (120 or 860 sampled species relative to over 9500 extant squamates) likely underestimates the actual number of evolutionary transitions in habitat that have occurred within most lineages. Despite this caveat, we estimated very few transitions between arid and mesic extremes, a result that was robust to differences in the size of the dataset and in the methods used to reconstruct transitions. This finding is broadly consistent with the idea that adaptation to arid (or mesic) extremes may often involve intermediate habitats as evolutionary "stepping stones." This propensity for evolution to proceed through semiarid intermediates could reflect the inherent challenges of adaptation between environmental extremes, a geographic bias due to the tendency for semiarid regions to bridge arid and mesic habitats in physical space, or (most likely) some combination of these factors. Although a direct quantification of the extent to which these factors interact to shape adaptation to habitat aridity is beyond the scope of our study, we note that the idea of "geographic bias" is supported by the observation that species ranges of North American squamates from semiarid habitats tend to border both arid and mesic extremes, whereas the ranges of arid and mesic species are much less likely to border on habitats of the opposite extreme (Fig. S1).

Despite the overall pattern of phylogenetic conservatism in habitat aridity, it is also clear that evolutionary transitions in habitat can occur fairly frequently, as we estimated approximately 124 transitions across a phylogeny of only 120 species, and 606 transitions across a phylogeny of 860 species. Not surprisingly, we documented significant phylogenetic signal in EWL, which is to be expected if this physiological trait is adapted to habitat aridity, which in turn is evolutionarily conserved. Though statistically significant, phylogenetic signal in EWL was fairly low ($K = 0.326$ for absolute EWL, 0.196 for mass-adjusted EWL), indicating relatively greater variance within clades than expected under Brownian motion. This is consistent with expectations for the adaptive evolution of traits under natural selection (Blomberg et al. 2003), and could also indicate adaptive phenotypic plasticity. It is generally inconsistent with the idea that phylogenetic signal in EWL is due to so-called phylogenetic inertia or evolutionary constraint.

This adaptive interpretation is corroborated by direct tests for an association between EWL and habitat aridity using data from extant species (i.e., nonphylogenetic analyses) and data accounting for phylogenetic nonindependence under several different frameworks (i.e., independent contrasts, phylogenetic

ANOVA, PGLS using Brownian motion and Ornstein–Uhlenbeck models of character evolution). In all comparisons, species from arid habitats exhibited substantially and significantly lower rates of EWL than their counterparts from mesic habitats (Fig. 1). Species from semiarid habitats exhibited intermediate rates of EWL, though they were statistically indistinguishable from those of species from arid habitats in our phylogenetic analyses (Fig. 1). Although we separated arid habitats (i.e., true deserts) from semiarid habitats (e.g., dry steppes, grasslands, dry forests), both are often considered together under the umbrella category of "drylands" (Dietz and Veldhuizen 2004) and share extended episodes of low precipitation and humidity that are punctuated by variable amounts of rainfall (Chesson et al. 2004; Loik et al. 2004). Physiological similarity in EWL between arid and semiarid species is therefore not surprising, and squamates from both arid and semiarid habitats clearly exhibit substantially lower levels of EWL than their mesic counterparts. These patterns corroborate earlier, nonphylogenetic comparative analyses of lizards (Mautz 1982aa, b). They also agree with more recent phylogenetic perspectives from birds (Williams 1996; Tieleman et al. 2003), mammals (Williams et al. 2004; Withers et al. 2006; Van Sant et al. 2012), and insects (Kleynhans and Terblanche 2009).

This broad physiological convergence among lineages is particularly striking given the many differences in behavior, ecology, morphology and physiology that distinguish these groups. For example, even when comparing (relatively) closely related lineages such as birds and squamates, we find a number of important differences with respect to thermoregulation, metabolism, behavior, and skin physiology, which interactively determine total EWL. Birds are endothermic and have substantially higher metabolic rates than squamates of comparable size (Pough 1980; Nagy 1987), which should dramatically increase their respiratory water loss, a component of total EWL. However, many birds also use cutaneous water loss as a primary means of thermoregulation via evaporative cooling (Williams and Tieleman 2005), whereas most squamates maintain body temperatures predominantly thorough behavioral adjustments (Huey and Slatkin 1976). The cellular and molecular arrangement of the epidermis and associated components of the skin barrier to cutaneous water loss also differ considerably among birds, reptiles, and mammals (Maderson 1972; Alibardi 2003; Lillywhite 2006). Nonetheless, the primacy of water conservation in arid habitats has led to consistent physiological convergence in whole-organism rates of EWL, both within and across these disparate lineages.

The mechanisms underlying this inferred physiological adaptation to habitat aridity could include a variety of factors impacting either respiratory water loss (e.g., metabolic rate) or cutaneous water loss (e.g., lipid barriers in the epidermis). Birds and marsupials from arid habitats often exhibit lower basal metabolic rate than their mesic counterparts (Tieleman et al. 2003; White 2003; Withers et al. 2006), although this pattern is not detected in all mammal lineages (Williams et al. 2004). Although the adaptive reduction of metabolic rate has been suggested for squamates in arid environments (Snyder 1971), we are not aware of any conclusive, large-scale test of this hypothesis. Alterations to the structure, vascularization, and lipid content of the epidermis are each associated with changes in cutaneous water loss in many vertebrates, including squamates (Elias et al. 1977; Roberts and Lillywhite 1983; Withers et al. 1984; Menon et al. 1996). In birds, these morphological and physiological adaptations have been linked to habitat aridity (Muñoz-Garcia and Williams 2005). However, metabolism and epidermal structure, along with their downstream effects on respiratory and cutaneous water loss, can also display a remarkable amount of developmental and phenotypic plasticity in response to temperature and aridity (Cox et al. 2008; Munoz-Garcia et al. 2008; Muñoz-Garcia and Williams 2008). The scope for plasticity is apparently greater in arid- than in mesic-adapted populations of the house sparrow, *Passer domesticus* (Muñoz-Garcia and Williams 2008), suggesting the interaction of genetic and environmental factors. This raises the important point that, although our results clearly show that evolutionary shifts in habitat aridity are consistently associated with predictable changes in EWL, our analyses cannot tease apart the relative contributions of genetic evolution (adaptation) and phenotypic plasticity (acclimation) in structuring this match between environment and physiology.

In summary, squamate reptiles exhibit an overall pattern of phylogenetic conservatism in both habitat aridity and EWL, but their historical colonization of arid and mesic habitats is repeatedly associated with changes in EWL that are likely adaptive. This physiological convergence, which may reflect a combination of phenotypic plasticity and genetic adaptation, has likely contributed to the ecological and evolutionary success of squamates in both arid and mesic environments. Though our data cannot directly address whether colonization and diversification are constrained by any difficulties inherent in these processes of physiological adaptation and acclimation, they do reveal that evolutionary transitions in habitat aridity are much more likely to occur through semiarid intermediates than directly between arid and mesic extremes, potentially due to an inherent tendency for the geographic ranges of semiarid species to border both arid and mesic habitats. Nonetheless, many squamate families contain a diversity of species arrayed across this spectrum from mesic to arid habitats, and these species show an adaptive pattern of correlated variation in their rates of total evaporative water loss.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. List of primary and secondary sources for estimates of total evaporative water loss and associated data for squamate reptiles in this study.
Table S2. Literature sources for habitat type for 860 species of squamate reptiles.
Table S3. Effects of biological predictor variables (habitat, body mass) and potential sources of among-study variance (measurement temperature, method of measurement) on rates of TEWL using non-phylogenetic linear models including interactions.
Table S4. Corrected Akaike Information Criteria (AICc) values for each model of character transition and for both EWL and expanded datasets.
Figure S1. Number and type of habitats that border the species ranges of North American squamate reptiles.