



Environment predicts repeated body size shifts in a recent radiation of Australian mammals*

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Closely related species that occur across steep environmental gradients often display clear body size differences, and examining this pattern is crucial to understanding how environmental variation shapes diversity. Australian endemic rodents in the *Pseudomys* Division (Muridae: Murinae) have repeatedly colonized the arid, monsoon, and mesic biomes over the last 5 million years. Using occurrence records, body mass data, and Bayesian phylogenetic models, we test whether body mass of 31 species in the *Pseudomys* Division can be predicted by their biome association. We also model the effect of eight environmental variables on body mass. Despite high phylogenetic signal in body mass evolution across the phylogeny, we find that mass predictably increases in the mesic biome and decreases in arid and monsoon biomes. As per Bergmann's rule, temperature is strongly correlated with body mass, as well as several other variables. Our results highlight two important findings. First, body size in Australian rodents has tracked with climate through the Pleistocene, likely due to several environmental variables rather than a single factor. Second, support for both Brownian motion and predictable change at different taxonomic levels in the *Pseudomys* Division phylogeny demonstrates how the level at which we test hypotheses can alter interpretation of evolutionary processes.

KEY WORDS: Adaptation, Bayesian phylogenetic multilevel models, Bergmann's rule, environmental gradient, Murinae, *Pseudomys*.

Closely related species that occur across steep environmental gradients often display clear phenotypic differences. Examining correlations between phenotypes and environment is crucial to understanding how environmental variation shapes functional diversity and facilitates speciation (Mayr 1956). Some phenotypic traits appear to be particularly labile, with the potential to shift dramatically at short evolutionary timescales (Lister 1989; Barnosky et al. 2003; Millien et al. 2006). Body size is a quantifiable and labile phenotypic trait (Woodward et al. 2005) that is strongly associated with many aspects of organismal life history, ecology, and behavior (Arendt 2007). Body size is

thought to be a frequent target of positive directional selection (Kingsolver and Pfennig 2004), and its evolvability can provide insight into the capacity of organisms to adapt to changing environments.

Parallel evolution of traits in response to similar environmental pressures is often used as evidence for adaptive evolution (Losos and Ricklefs 2009; Salzburger 2009; Elmer et al. 2010; Elmer and Meyer 2011; Losos 2011). If selection on body size favors different optima under distinct climatic conditions, then we expect body size to vary across environmental gradients, both within and between species. Bergmann's rule is a frequently invoked ecogeographic generalization that states that homeothermic animals at higher latitudes or in colder climates will be larger than their close relatives living at lower latitudes and in warmer climates (Bergmann 1847; Mayr 1963). This pattern is thought

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to confer a selective advantage to the challenges of thermoregulation (Mayr 1956). Bergmann's rule has been applied at both intraspecific and interspecific taxonomic levels and across local and global scales. Empirical studies have confirmed that body size in homeothermic animals frequently varies with both latitude and temperature, with a review by Meiri and Dayan (2003) finding that 65% of studied mammal species and 72% of studied bird species conform to Bergmann's rule. However, several studies have questioned the validity and generality of this ecogeographic rule (Geist 1987; Ashton et al. 2000). Although Bergmann's rule is most commonly associated with temperature gradients, other correlated environmental variables, such as precipitation and moisture index, may also drive body size disparity (Millien et al. 2006). The more recently proposed "resource rule" (sensu McNab 2010; Huston and Wolverton 2011) suggests that larger body size is a consequence of greater resource availability (e.g., food sources and water) in more productive regions. Although most environmental variables broadly associate with latitude on a global scale, local scale environmental gradients are often more complex. Assessing patterns of body size variation in young clades across highly heterogeneous environments may reveal parallel variation that is not evident on global scales, and that have not been distorted through deep evolutionary time.

The heterogeneous environments of Australia can be broadly categorized into arid, monsoon, temperate mesic, and tropical rainforest biomes (Olson et al. 2001; Byrne et al., 2008, 2011; Bowman et al. 2010; Catullo and Keogh 2014). Although annual mean temperature in Australia follows a latitudinal gradient to an extent, other environmental axes (e.g., precipitation) vary primarily by biome. As such, Australian environments are best characterized using a combination of climatic variables. Perhaps the steepest ecological gradient in Australia is the transition from coastal regions to the arid interior, which is strongly delineated by differences in aridity, temperature range, and precipitation. On a global scale, arid environments have been considered a diversity sink (Crisp et al. 2009), as it is assumed that specialized physiological and morphological adaptations are required to exploit such harsh environments. However, recent studies have identified the aridification of Australia as a driver of diversification (Rabosky et al. 2007; Fujita et al. 2010; Catullo and Keogh 2014; Mitchell et al. 2014; Brennan and Oliver 2017; Cardillo et al. 2017; Smissen and Rowe 2018). Some groups, such as rodents (Smissen and Rowe 2018), appear to readily transition bidirectionally across extreme environmental gradients. This suggests that the exploitation of the arid biome may be less challenging than expected, and that traits associated with arid and mesic biomes may not be as evolutionarily constrained as predicted.

The endemic Australian rodents are recent and successful colonizers of almost all Australian habitats. The *Pseudomys* Division (following Smissen and Rowe 2018), including the

genera *Pseudomys*, *Mastacomys*, *Notomys*, *Leggadina*, and *Zyomys* (Hydromyini: Murinae: Muridae: Rodentia), is the primary radiation of endemic Australian murine rodents. Their diversity (41 historically extant species) accumulated rapidly and recently, having colonized Australia and radiated from a single ancestor within the last 5–6 million years (Rowe et al. 2008; Smissen and Rowe 2018; Roycroft et al. 2019). Species in the *Pseudomys* Division are distributed across all biomes in Australia except the tropical rainforest, and appear to be exceptionally flexible with respect to their habitat association. Contrary to expectations from niche conservatism, Smissen and Rowe (2018) reconstructed 12 speciation events associated with transitions between disparate biomes, largely during the climatically unstable Pleistocene. Transitions between biomes are concentrated within the clade comprising the genera *Pseudomys* and *Mastacomys*, with multiple, recently diverged arid-mesic sister species pairs (Fig. 1). This phylogenetic framework provides an opportunity to link possible instances of parallel evolution of phenotypic traits among nonsister taxa inhabiting the same environment.

Across the *Pseudomys* Division, and within the genera *Pseudomys* and *Mastacomys* alone, there is at least a 15-fold variation in interspecific body mass, ranging from ~8 g (*Pseudomys delicatulus*) to ~120 g (*Mastacomys fuscus*) (Breed and Ford 2007). This variation in body mass may be the result of selection toward different fitness optima in disparate biomes, for example, through conferring advantages in thermoregulation or water conservation (Gür and Kart Gür 2012; Giorello et al. 2018). Using occurrence data, body mass as a measure of body size, and Bayesian phylogenetic multilevel modeling, we test whether body size in the *Pseudomys* Division can be predicted by their biome association and by continuous environmental variables. If variation in body size is the result of differing selective pressures in disparate biomes, we expect that variation in body mass will track biome occupancy. Given the heterogeneous nature of Australian landscapes, we predict that temperature may not be the primary correlate to body size variation, in contrast to expectations from Bergmann's rule.

Materials and Methods

OCCURRENCE DATA, BODY MASS, AND ENVIRONMENTAL VARIABLES

We obtained occurrence data for 31 species of murine rodents in the *Pseudomys* Division from the Atlas of Living Australia (ALA, <https://www.ala.org.au/>). We included all records of each species that corresponded to preserved specimens, material samples, or observations in biodiversity surveys, and excluded all paleontological records. To remove any misidentified occurrences and databasing errors, we filtered any records that fell outside the known range of the species using range boundaries taken from the

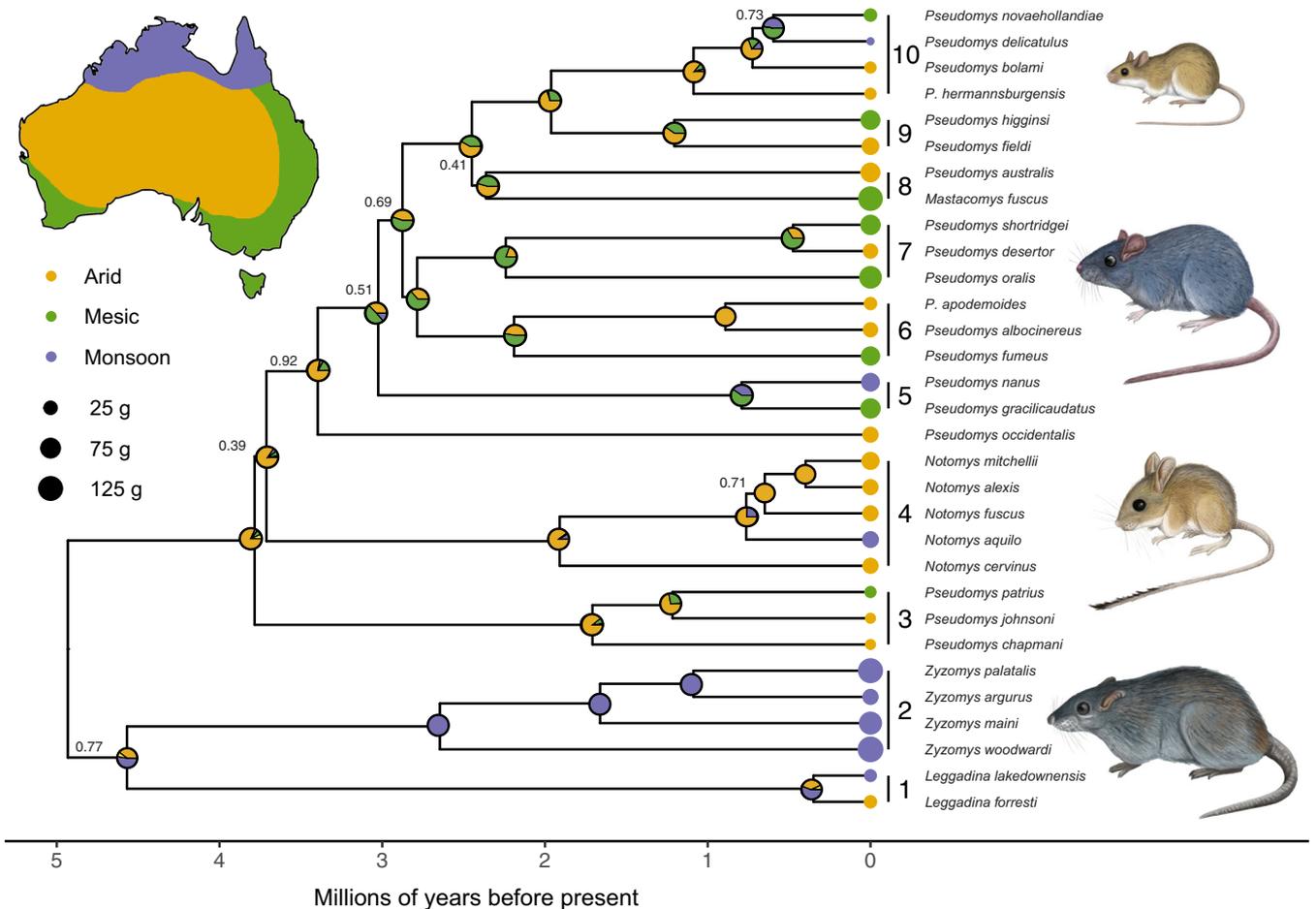


Figure 1. Time-calibrated maximum clade credibility phylogeny of the Pseudomys Division (Smitsen and Rowe 2018). Nodes with posterior probability <0.95 are labeled. Circles at each tip are colored by biome (indicated on inset map) and are scaled to mass (grams) by area. Pie charts on internal nodes are modified from ancestral state reconstruction reported in Smitsen and Rowe (2018). An additional fourth state (light yellow) indicates the potential for ancestral codistribution in arid and monsoon biomes at the most recent common ancestor (MRCA) of *Leggadina forresti* and *L. lakedownensis*, the MRCA of *Leggadina* and *Zyzomys*, and the MRCA of subclades 3–10. Ancestral nodes are not scaled to body mass. Subclades used in the analysis are labeled 1–10. Illustrations (top to bottom) of *Pseudomys delicatulus*, *P. fumeus*, *Notomys alexis*, and *Zyzomys woodwardi* by Subir Shakyia.

IUCN Red List 2008 (available at <http://www.iucnredlist.org/>). Average body mass (in grams) for each species was taken from Breed and Ford (2007) (Supporting Information Appendix I). For each occurrence point, we obtained 19 environmental variables (average of years 1976–2005) from ALA, using data derived from ANUCLIM (Xu and Hutchinson 2011). For each species, we then took the mean and standard deviation of each variable across all occurrence points. To avoid collinearity of environmental variables, we performed variable reduction by generating a correlation matrix for the entire dataset. All variables that were highly correlated ($>80\%$ correlation coefficient) with temperature, precipitation, and aridity were removed, leaving *temperature (annual mean)*, *temperature range (annual mean)*, *precipitation (annual mean)*, *precipitation (annual seasonality ratio)*, *aridity index (annual mean)*, and *elevation* (see Table S1 for definitions). The

retained variables broadly represent the environmental heterogeneity in Australian landscapes. We also included *net primary productivity (annual mean)* and latitude to directly test both the “resource rule” (McNab 2010) and Bergmann’s rule (Bergmann 1847; Mayr 1963). We additionally classified species into one of three biome categories based on the biome classifications of Smitsen and Rowe (2018). Species were assigned to each biome if they had $>75\%$ of their geographic distribution falling within that biome (as defined in Olson et al. 2001; Byrne et al., 2008, 2011; Bowman et al. 2010; Catullo and Keogh 2014).

TESTING FOR BODY SIZE CHANGES BETWEEN BIOMES

We used Bayesian phylogenetic multilevel models to first determine whether body mass of species in the Pseudomys Division

could be predicted by their biome association. We used the time-calibrated, multispecies coalescent phylogeny of Smissen and Rowe (2018), generated using StarBEAST2 (Bouckaert et al. 2014; Ogilvie et al. 2017), along with mean body mass (Breed and Ford 2007) and biome classifications (arid, mesic, or monsoon) for each species (Fig. 1; Smissen and Rowe 2018). All models were fit with Stan (Carpenter et al. 2017) using the R package brms (Bürkner 2017). We used linear models with the natural log of body mass as the dependent variable, biome categories (arid, mesic, and monsoon) as the predictor variables, and a species level matrix of scaled phylogenetic branch lengths (i.e., the phylogenetic correlation matrix; Bürkner 2017) as a group-level effect (de Villemereuil et al. 2012). To account for phylogenetic uncertainty in the *Pseudomys* Division topology, we used custom scripts to summarize over 100 randomly sampled trees from the posterior distribution of trees generated by Smissen and Rowe (2018). Regression models with categorical predictor variables treat one category as the intercept value (i.e., “dummy variable,” Bürkner 2017). We therefore removed the intercept parameter from the model so that the posterior distribution of the phylogenetically corrected log-mass was estimated directly for each group. We fit regularizing normal priors on the population-level effects ($N(0, 1.5)$) to prevent MCMC chains from occasionally searching very large, unreasonable values of model space (Gelman 2006; McElreath 2016). We used the Student's t distribution to describe the response variable, which is insensitive to outliers (Kruschke 2013; Bürkner 2017). Each of the 100 models included four chains run for 5000 generations, with 2500 generations of warm-up and 2500 chains of sampling, resulting in 1,000,000 posterior samples. This method allowed us to calculate the phylogenetic signal (Pagel's λ ; Pagel 1994) of body mass using a mixed-model approach (Housworth et al. 2004; Hadfield & Nakagawa 2010; Bürkner 2017). To determine if the corrected estimates of body mass differed between biomes, we calculated the difference between the posterior distributions of body mass from each biome (arid–mesic, arid–monsoon, monsoon–mesic). If the 95% credible interval of these difference distributions does not overlap zero, then we reject that body mass is equal between biomes, an approach analogous to the “Bayesian robust t-test” proposed by Kruschke (2013).

CONTINUOUS ENVIRONMENTAL VARIABLES AND BODY SIZE

As Australian environments are highly heterogeneous, and species do not all strictly fall exclusively within biome boundaries, we tested whether variation in body mass among *Pseudomys* Division species could be explained by variation in continuous environmental variables using linear Bayesian phylogenetic multi-level models. To avoid oversimplifying the environmental niche for each species, many of which have broad distributions across much of the continent, we included both the mean and standard

deviation of each continuous predictor variable by using the measurement error term $me(x_1, sdx_1)$ in the *brm()* model function in brms (Bürkner 2017). Therefore, our species-level estimates of slope and intercept include the effects of environmental heterogeneity across the range of each species. As noted above, many recently diverged sister lineages in the *Pseudomys* Division inhabit different biomes. We leveraged this pattern by partitioning the tree into 10 subclades (see Fig. 1) and used the clade number as a group-level effect in the models. As *Pseudomys occidentalis* is on a long branch and cannot, at present, be assigned to a recent clade, it was excluded from these analyses. Our multi-level models estimate an individual slope and intercept for each group (clade in our case) and then summarize over these estimates to generate a posterior distribution of the effect of the response on the predictor (McElreath 2016). We used the phylogenetic correlation matrix as a second group-level effect. Each variable was run in an independent model to avoid the effects of multicollinearity. All climatic variables were log-transformed and mean-centered on zero to improve model mixing, provide a conservative and more interpretable result, and eliminate multiple comparison issues (Gelman and Tuerlinckx, 2000). Variables were log transformed prior to estimating their standard deviation. We also ran interaction models to determine whether the additive effects of multiple environmental variables better explain variation in body size. Each model included four chains run for 5000 generations, with 2500 generations of warm-up and 2500 chains of sampling. Model convergence was assessed using the \hat{R} potential scale reduction factor (Brooks and Gelman 1998). In summary, these models allowed us to incorporate phylogenetic variance, biome occupancy, continuous environmental variables and their variability, and potential interaction effects for each species. Data and scripts for all analyses are available on GitHub (https://github.com/jonnations/Pseudomys_body_size).

Results

BODY SIZE CHANGES BETWEEN BIOMES

All models of the effect of body mass on habitat categories (biomes) converged, and all parameters had high effective sample sizes (>2000) and low \hat{R} values (≤ 1.01). By comparing the differences of the posterior distributions of phylogenetically corrected log-body mass among biomes (Fig. 2, Table 1), we found that species from the mesic biome are larger than those from either arid or monsoon biomes (i.e., the 95% credible intervals of the differences between the arid–mesic and monsoon–mesic comparisons posterior distribution did not overlap zero). In contrast, we found no difference in body mass between the arid and monsoon posterior distributions. The posterior distribution of Pagel's λ values showed an HPD of 0.995 and a mean of 0.94, indicating strong phylogenetic signal and suggesting that body mass has evolved according to a Brownian motion model.

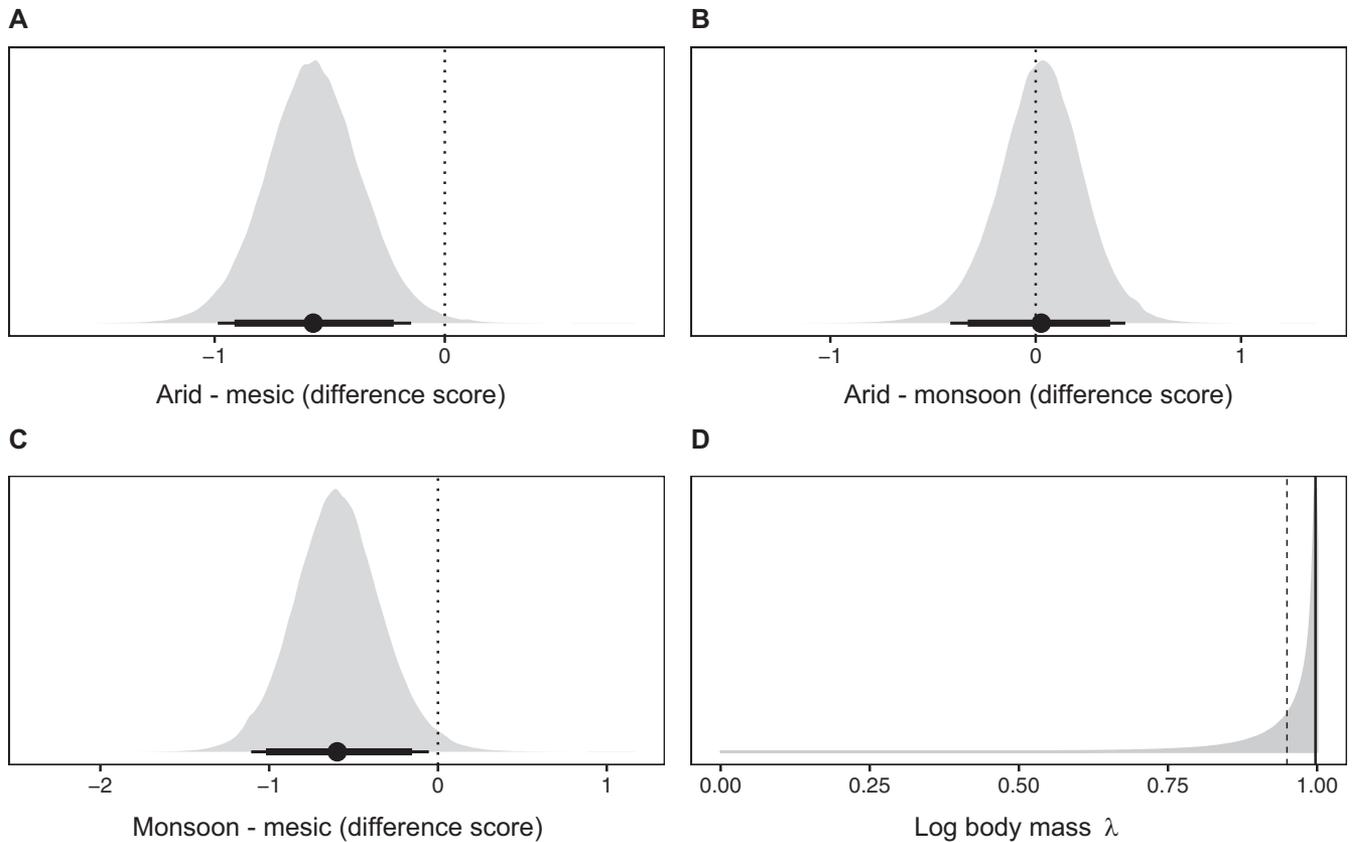


Figure 2. Differences in posterior distribution of phylogenetically corrected log body mass between (A) arid and mesic biomes, (B) arid and monsoon biomes, and (C) monsoon and mesic biomes. Dotted vertical lines represent zero, that is, zero difference between the posterior probabilities, horizontal bars represent the 90% and 95% credible intervals and black circles represent the highest posterior density of each distribution. (D) Posterior distribution of Pagel's lambda for log-transformed body mass. The solid vertical line shows the highest posterior density, and the dashed line indicates the mean.

Table 1. Results from Bayesian phylogenetic multilevel models of categorical biome groupings (upper) and continuous environmental variables (lower), where α represents the intercept and β represents the regression coefficient.

Categorical biome grouping	α Mean	α 95% CI	β Mean	β 95% CI
Arid	—	—	2.99	(2.17, 3.69)
Mesic	—	—	3.56	(2.68, 4.32)
Monsoon	—	—	2.97	(2.12, 3.70)
Environmental variable				
Temperature (annual mean)	3.67	(3.05, 4.29)	−1.74	(−3.42, −0.36)
Temperature range (annual mean)	3.67	(3.07, 4.30)	−1.61	(−3.59, 0.20)
Precipitation (annual mean)	3.59	(2.96, 4.20)	0.32	(−0.05, 0.76)
Precipitation (annual seasonality ratio)	3.60	(2.92, 4.30)	−0.15	(−0.54, 0.25)
Net primary production	3.64	(3.03, 4.26)	0.27	(−0.01, 0.55)
Elevation	3.54	(2.64, 4.33)	0.51	(−2.76, 4.76)
Aridity index (annual mean)	3.60	(2.99, 4.22)	0.22	(−0.01, 0.47)
Latitude	3.61	(2.94, 4.29)	−0.02	(−0.07, 0.02)

INFLUENCE OF ENVIRONMENTAL VARIABLES, AND THEIR INTERACTIONS, ON BODY SIZE

We tested the effect of eight continuous variables and 12 variable interactions on body mass across the *Pseudomys* Division using

phylogenetic relationship and subclade membership as group-level effects. All models converged and had high effective sample sizes (>2000) and low \hat{R} values (≤ 1.01). At a 95% credible interval, we found that temperature (annual mean) had a strong

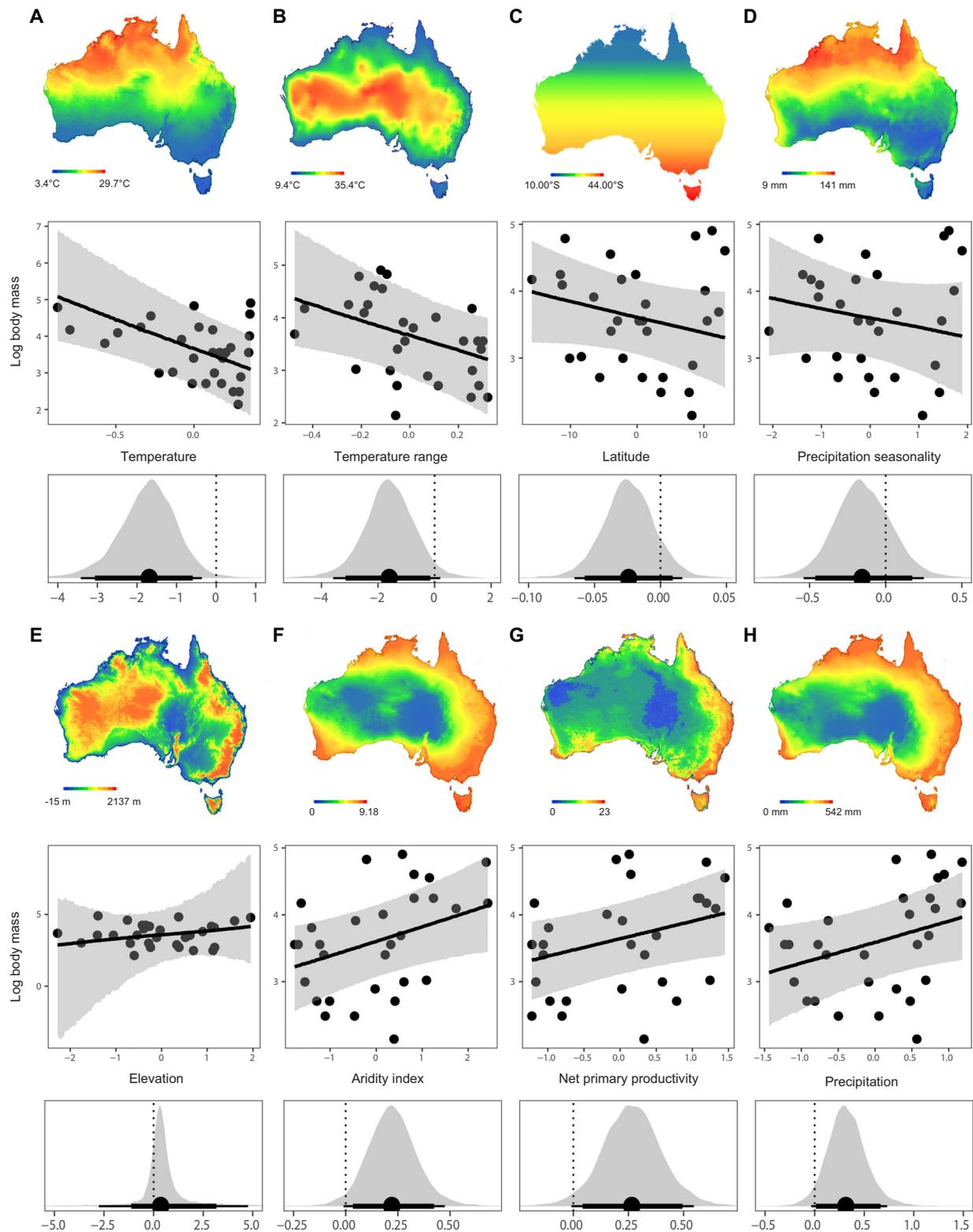


Figure 3. Bayesian phylogenetic multilevel regression of body mass and environmental variables. For each panel, the top image shows the values of each variable across Australia; the middle plot shows the log-transformed, mean-centered environmental variable values (x-axis), log body mass (y-axis), regression line, and credible intervals, and the lower plot shows the effect (i.e., slope) posterior distributions for (A) temperature (annual mean), (B) temperature (annual range), (C) latitude, (D) precipitation (annual seasonality ratio), (E) elevation, (F) aridity index (annual mean), (G) net primary productivity, and (H) precipitation (annual mean). On each lower plot, horizontal bars represent the 90% and 95% credible intervals and black circles represent the highest posterior density of each distribution.

negative effect (β mean = -1.74) and was credibly nonzero, and at a 90% credible interval, temperature (range), precipitation (annual mean), net primary productivity, and aridity were nonzero (Table 1, Fig. 3). Precipitation and net primary productivity had moderately positive effects, with β mean = 0.32 and 0.22 , respectively (Table 1). Elevation, precipitation (annual seasonality ratio), and latitude had little to no effect on body mass (Fig. 3). Models that included interactions between variables had a smaller effect than all eight independently tested variables (Table S2), indicating that differences in body size were not better explained by additive effects of multiple environmental variables.

Discussion

Rodents of the *Pseudomys* Division have radiated across Australia over the past 5–6 million years, and presently inhabit almost all environments in this heterogeneous landscape. Within the *Pseudomys* Division, repeated transitions across biome boundaries have resulted in multiple clades occupying widely divergent environments. For the *Pseudomys* Division as a whole, body mass has evolved in a manner consistent with a Brownian motion model. However, we also found that body mass is tightly correlated with environment, and that species that inhabit mesic environments are consistently larger than their close relatives found in arid or monsoon biomes (Fig. 2, Table 1). These differences in body size have evolved repeatedly and predictably over the past 2.5 million years. The climatic differences among the temperate mesic, monsoon, and arid biomes of Australia are striking, and our results are consistent with the hypothesis of environment as a driver of parallel body size evolution in mammals.

DIRECTION AND FLEXIBILITY OF BODY SIZE EVOLUTION

The direction and evolutionary flexibility of trait evolution can provide insight into how species adapt to novel and changing environments. Predictable and repeated examples of body size shifts associated with biome transitions in the *Pseudomys* Division (Fig. 1) suggest that endemic Australian rodents are not limited by niche conservatism, especially within the *Pseudomys* genus. In the case of sister species *Pseudomys desertor* (30 g) and *Pseudomys shorridgei* (70 g), the shift in body mass is particularly noticeable (Fig. 1). Although ancestral reconstruction indicates that the clade of *Notomys* + *Pseudomys* + *Mastacomys* (subclades 3–10; Fig. 1) originated from an arid-distributed ancestor, *P. desertor* and *P. shorridgei* are predicted to have diverged from a mesic-distributed ancestor (Fig. 1, Smissen and Rowe 2018). As such, our results suggest that *P. desertor* decreased in body size as a result of recolonizing the arid biome within the last 500,000 years. Other sister clade divergences appear to have

originated from arid ancestors, for example, the delicate mouse clade (*Pseudomys hermannsburgensis*, *Pseudomys bolami*, *Pseudomys delicatulus*, and *Pseudomys novaehollandiae*). This suggests that the mesic-distributed *P. novaehollandiae* underwent a body size increase following colonization of the mesic biome, and indicates that body size, much like biome occupation, is bidirectionally flexible. Evolutionary flexibility in key traits like body size was likely integral in the ability of the *Pseudomys* Division to succeed in a variety of environmental conditions. A possible alternative hypothesis to explain rapid body size shifts in the *Pseudomys* Division is that competition among sympatric species has driven ecological character displacement (Brown and Wilson 1956; Losos 2000). However, body size differences in endemic Australian rodents are not likely driven by competition with close relatives, as sister species (or members of the groups as defined in our analyses) do not generally co-occur. Although the distribution of a small number of species in the *Pseudomys* Division span biome boundaries (e.g., *Zygomys argurus* and *P. delicatulus*), the clear pattern we recover using three state categorization suggests a robust pattern at the species level. Size variation has also been reported at an intraspecific level within *P. delicatulus* and *P. hermannsburgensis* (Ford 2003; Tomlinson and Withers 2008); however, body mass data are not available at distribution-wide scale for most species. Further studies of trait variation within species could provide insight into intraspecific variation across the landscape, as well as the evolvability of native rodents in the face of future changing climates.

COMPLEX ENVIRONMENTAL CORRELATES

We tested the influence of environmental variables on body mass evolution in the *Pseudomys* Division and found the strongest relationship between body mass and annual mean temperature, but also found support for the influence of annual temperature range, precipitation, aridity, and net primary productivity (Fig. 3, Table 1). Many of these variables are moderately correlated across the Australian landscape, and it is not surprising to find support for more than one. Several previous studies have highlighted that body size variation is often associated with a complex of interacting environmental factors, rather than a single driver (Hamilton 1961; Mayr 1963; Yom Tov and Nix 1986; Millien et al. 2006). Given this pattern, it is interesting to note that our models integrating the additive effects of continuous environmental variables did not better explain body mass variation than did any single variable (Table S2). Although body mass varies along a temperature gradient in the *Pseudomys* Division, a meta-analysis across mammals suggested that rodents are among the only mammalian exceptions to Bergmann's rule (Meiri and Dayan 2003). In contrast, a more recent, integrated study across Rodentia recovered a strong correlation between precipitation and body size (Alhajer and Steppan 2016). Similarly, our results support a pattern

of complex environmental correlates to body size in rodents, but that temperature is a strong driver, as per Bergmann's rule. Interestingly, we found that latitude was a poor predictor of body size. This suggests that the use of latitude as a proxy for complex environmental variables may be misleading, especially in Australia where environmental heterogeneity falls on a coastal to interior gradient. A previous Australian study did not find evidence for Bergmann's rule in *Pseudomys hermannsbergensis* across its primarily arid distribution, and recovered a pattern where individuals were larger in more arid regions (Tomlinson and Withers 2008). This is in contrast to our results, and may suggest that recent, intraspecific variation is the result of different selective pressures to that of broader scale, interspecific variation.

CAUSES AND FUNCTIONAL CONSEQUENCES OF BODY SIZE TRANSITIONS

A classic mechanistic explanation for associations between temperature and body size is that larger size in colder environments affords thermoregulatory benefits due to a smaller surface-to-volume ratio (Bergmann 1847; Mayr 1956, 1963). Conversely, a higher surface-to-volume ratio may enable more efficient heat dissipation in warmer environments (Brown 1968). In contrast, the "resource rule" (sensu McNab 2010; Huston and Wolverton 2011) suggests that larger body size is a consequence of greater resource availability (e.g., food sources and water) in more productive regions. In temperate regions of Australia, areas with high primary productivity tend to have higher rainfall and cooler temperatures, making these factors difficult to independently assess. Interestingly, we found that *Pseudomys* Division species in both the arid and monsoon biome were significantly smaller than their mesic counterparts (Fig. 2), and that precipitation seasonality did not significantly correlate with body size (Fig. 3d). Monsoon habitats are best defined by seasonality in rainfall, fluctuating annually between periods of extreme aridity and periods of intense rainfall. Species that inhabit the monsoon biome must be capable of tolerating both these extremes. Our results suggest that the physiological challenges of the dry season in monsoon environments are likely a primary limiting factor to body size in Australian murines.

Overall, the results of our environmental variable regression analyses suggest that a combination of factors facilitated body size transitions in the *Pseudomys* Division. For example, following initial colonization of the mesic biome, an ancestral population may have experienced thermoregulatory-related selection for size increase on standing intraspecific body size variation, which was then reinforced by the greater abundance of resources. In addition to body size, a suite of other physiological and behavioral traits often covary with environment, including insulation (Briscoe et al. 2015) and basal metabolic rate (Fristoe et al. 2015). Specifically among rodents, kidney function (MacMillen

and Lee 1967; Giorello et al. 2018), diet (Degen et al. 1986), burrowing (Walsberg 2000), and sociality (Ebensperger 2001) are likely often associated with environment, particularly as adaptations to arid environments. Species in the *Pseudomys* Division are understudied with respect to many aspects of their ecology, physiology, and behavior. Increased future focus on the ecology of these species will help elucidate other associated traits important in transitions between arid and mesic environments.

INTERPRETING HIERARCHICAL PATTERNS OF TRAIT EVOLUTION

Body size evolution across the *Pseudomys* Division showed a high level of phylogenetic signal (Pagel's λ , Fig. 2d), suggesting that this trait follows a Brownian motion model of evolution across the group as a whole (Pagel 1994). However, the results of our phylogenetic body mass estimates found that mass tracks with recent biome transitions in a nonrandom manner (Fig. 2a–c), which, initially, appears to contrast our findings of high phylogenetic signal. An alternative explanation may explain this discrepancy. Recent work has highlighted that size-related trait evolution may take place closer to the present, at the tips of a phylogeny, as species adapt to modern habitats (Slater and Friscia 2019), a pattern also recovered in the *Pseudomys* Division. We hypothesize that the high phylogenetic signal is due to fluctuating selective pressures on body size in changing environments. Fluctuating selection is shown to increase phylogenetic signal when the rate of movement of the optimum phenotype is high (Revell et al. 2008). Australia underwent significant climatic oscillations throughout the Pliocene and Pleistocene (Byrne et al., 2008, 2011), affecting traits, distributions, and diversification of contemporary species (Potter et al. 2018). During this time, species in the *Pseudomys* Division repeatedly colonized the expanding and contracting arid, monsoon, and mesic biomes (Smitsen and Rowe 2018), and the optimum body size of species in the *Pseudomys* Division shifted in response to these transitions, resulting in a high λ value. Environmental history, therefore, provides a simple explanation for a contrasting pattern of randomness and predictability. These results demonstrate that interpretations of evolutionary process may vary depending upon which hierarchical level of the phylogeny they are interrogated. This has important implications, especially for studies at broad taxonomic scales, in which evolutionary processes near the tips may be masked when analyzed in a global comparative context. Our finding of biome-driven shifts in body size highlights the importance of local patterns of phenotypic evolution when examining macroevolutionary processes at lower phylogenetic levels.

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AUTHORS CONTRIBUTIONS

EJR and JAN collated the data, performed the analyses, and wrote the manuscript. All authors conceived the study and contributed to the final manuscript.

DATA ARCHIVING

All data and custom scripts used in this manuscript have been made available on GitHub at https://github.com/jonnations/Pseudomys_body_size and all data used are provided in the Supporting Information Materials.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Table 1: Definitions of continuous variables used in analyses

Supplementary Table 2: Results from Bayesian phylogenetic multilevel models of interactions between continuous environmental variables