

## LETTER

# Phenotypic population divergence in terrestrial vertebrates at macro scales

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### Abstract

Phenotypic divergence between populations, i.e. how much phenotypes within a species vary geographically, is critical to many aspects of ecology and evolution, including eco-geographical trends, speciation and coexistence. Yet, the variation of divergence across species with different ecologies and distributions and the relative role of adaptive causes remains little understood. We predict that genetic control vs. phenotypic plasticity of traits, geographical distance and (assuming adaptation) environmental differences should explain much of the phenotypic variability between populations. We tested these predictions with body sizes of 1447 populations in 98 terrestrial vertebrate species. Population phenotypic variability differs strongly across species, and divergence increases with increasing levels of clade-typical phenotypic plasticity, the area covered by populations and body size. Geographical distance and environmental dissimilarity are similarly important predictors of divergence within species, highlighting a potential role for biotic and environmental conditions. Increased availability of phylogeographical and ecological data should facilitate further understanding of population divergence drivers at broad scales.

### Keywords

Amphibians, birds, body size, environment, gene flow, intraspecific variation, mammals, population variability, range size, reptiles, vertebrates.

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## INTRODUCTION

Populations of the same species can exhibit marked variation in morphology, physiology, life history traits and particularly body size (Mayr 1963; Yablokov 1966; Endler 1977). Such variation has long been of interest to ecologists and evolutionary biologists, especially for understanding the role of adaptation and natural selection (Gould & Johnston 1972; Endler 1977). Associations between phenotypic variation and environmental features suggest adaptive divergence among populations (Mayr 1963). This divergence in turn is the necessary raw material for population differentiation leading to speciation and provides the mechanistic basis for eco-geographical trends, e.g. a potential increase in mean body sizes of populations towards higher latitudes (Bergmann 1847; Ashton 2002; Meiri & Dayan 2003). Capturing the variability in mean phenotypes across multiple populations requires data across large geographical scales. As a result, even though such cross-population phenotypic heterogeneity is at the heart of

ecology and evolution, its variation across multiple species has received surprisingly limited general scrutiny.

We here define populations as geographical collections of individuals which form genetically distinct entities that evolve differently from other such units (Ehrlich & Daily 1993). We hypothesize that observed levels of population phenotypic divergence across species are affected jointly by (1) their geographical distribution and the associated environmental heterogeneity and spatial separation encountered by populations, (2) by the typical levels of per-distance gene flow between populations and (3) by the relative strengths of natural selection and phenotypic plasticity as they pertain to a given trait. For any trait under genetic control geographical variation across populations may arise simply from genetic drift (and be neutral). The effects of genetic drift are strongest across small fragmented populations (Lande 1980), but also act in continuously distributed populations if gene flow is restricted. Given that genetic distance tends to increase with geographical distance (Sokal 1983; Hutchison & Templeton 1999; Storz 2002;

Ramachandran *et al.* 2005), albeit modified by landscape structure (Manel *et al.* 2003), geographical distance should be an adequate proxy for gene flow.

If an organismal trait is tightly linked to fitness components that are associated with local conditions, cross-population variation is expected as a consequence of local adaptation or phenotypic plasticity. Species that span a wide range of environments would be expected to show more variable organismal traits (Mayr 1963; Meiri *et al.* 2007). In this case, a positive relationship between variation in organismal traits and geographical range size would arise due to the greater environmental variation encountered over a larger geographical extent. In the case of strongly plastic traits (e.g. adult body size in indeterminate growers), population responses may simply be a signal of phenotypic plasticity. In the cases of adaptation under natural selection, again levels of gene flow play an important additional role (Mayr 1963). Theory shows that when gene flow is pervasive and selection is not very strong, local adaptation, and, thus inter-population trait variation, is limited because genes from populations in other environments impede the evolution of environment-specific traits (Felsenstein 1976; Slatkin 1985). On the other hand, if gene flow is minimal, or selection strong, local adaptation is more likely to evolve because each population represents a fully or mostly isolated gene pool (Slatkin 1985). *Ceteris paribus*, species with populations that are widely separated, such as those with large geographical ranges, should have relatively larger trait variation due to their more limited total gene flow and greater genetic isolation. The geometry of the geographical range may be of significance, as in more circular ranges populations are on average closer to each other than elongated ranges. Given the existing but debated support – at least in homeotherms – for environmental gradients in trait variation in e.g. body mass (Bergmann's rule; Bergmann 1847; Scholander 1955; Ashton & Feldman 2003; Freckleton *et al.* 2003; Meiri & Dayan 2003; Meiri *et al.* 2007), environmental variation rather than spatial distance between populations may be expected to exert the stronger influence on phenotypic variability in this trait.

Gene flow is not only a function of distance between populations but also depends on the species-typical levels of genetic exchange between individuals, which is affected by the encounter frequency of individuals across landscapes. For a given distance this frequency should vary by species' mobility or dispersal ability and their abundance. While on a gradient from small to large-bodied species locomotion generally becomes energetically cheaper and dispersal distance larger (Calder 1984; Sutherland *et al.* 2000; Jenkins *et al.* 2007) – particularly in flyers (Alexander 1998) – abundance decreases with increasing body size more steeply (Damuth 1981). As a consequence, at least at landscape scales encounter frequencies are likely to decrease with

increasing body mass (Jetz *et al.* 2004). We speculate that then the overall result is lower gene flow and higher levels of phenotypic divergence in large-bodied species, but are unaware of tests of this idea.

Vertebrates are a useful model system to understand the relative importance of these factors, as they are ecologically diverse and show strong variability along all the major axes that we outlined, which may determine phenotypic population divergence. The sizes and shapes of their geographical ranges vary dramatically from several hectares to almost the whole globe – they occupy strongly differing environments, body masses vary by over 6 orders of magnitude among species and body mass determination differs between highly plastic in indeterminate growing clades to strongly genetically constrained in homeotherms (Sebens 1987).

We make the following predictions: (1) species that are predominantly indeterminate growers (such as amphibians, squamates and turtles) should show greatest body mass variability, as their plastic response may react to even small differences in local conditions between populations; (2) wide-ranging species are likely to encounter greater environmental heterogeneity and less gene flow and should therefore show greater phenotypic variability; (3) a presumed lower broad-scale genetic connectivity may lead to larger variability in large-bodied species; (4) both geographical distance (through its effect on gene flow) and environmental heterogeneity (through local adaptation or plasticity) between populations should contribute to phenotypic variability; but given the evidence for Bergmann's rule type latitudinal patterns, environmental effects should be stronger; (5) geographical distance via its effect on gene flow may be less important in indeterminate growers with their plastic response to environmental differences.

In this study we document broad-scale divergence in body mass and test purported relationships for 1447 populations across 98 species of terrestrial vertebrates worldwide. We examine trends within and across the five major clades: birds, mammals, chelonians (turtles), squamates (lizards and snakes) and amphibians.

## METHODS

### Body mass

We compiled average adult body mass information (in grams) for 1447 populations of 98 tetrapod vertebrate species from a total of 240 studies (see electronic Appendix S1, Fig. S1, Tables S1 and S5). Five clades (predictor: *Clade*) are represented: Amphibia (18 species, 247 populations), Aves (28 species, 750 populations), Chelonia (13 species, 134 populations), Mammalia (15 species, 158 populations) and Squamata (24 species, 158 populations). On average data for 14.8 populations

(SD = 14.2; range 3–76) were available per species (predictor:  $N$ ). For species with sexual size dimorphism the average population body mass was calculated as the average of population male and female body mass. If a study did not provide direct measures, we estimated body mass based on associations found in the literature between the reported body size metric and body mass. For 18 of the 98 species we relied on size metric–body mass relationships for congeneric species because the relationship was not available for the focal species. The results remain qualitatively unchanged, when measurement type (direct vs. indirect measurement) was controlled for (Tables S3 and S4). We  $\log_{10}$ -transformed body mass values to address the right skew found in the data and that is also typical of species-level body size frequency distributions (Brown & Maurer 1989), and calculated average species body mass (predictor:  $Mass$ ). We then estimated variability in body mass among populations of a species, the response variable of the first part of our analyses, as the standard deviation (SD) of  $\log_{10}$  body mass. For comparing relative size variability among groups of different sizes the SD of log-transformed data is strongly preferable to the coefficients of variation of raw data which are statistically problematic (Lewontin 1966).

Finally, we also compiled, for each species, a dissimilarity matrix of differences in body mass for all unique pair-wise combinations of populations.

### Geographical distribution

We geo-referenced all population locations manually using the information provided in the original sources and online digital gazetteers. Populations show a worldwide distribution (Fig. S1), with a bias towards North America and Europe. We also acknowledge that the data are not a random subset of vertebrate species, but have some taxonomic groups (e.g. rodents) represented more than others (see Tables S1 and S5). We estimated the size of the region sampled by populations for each species (predictor:  $Area$ ) using the area contained within a convex hull. The convex hull for a species was defined as the minimum convex set of population geographical coordinates that contained all the population locations. To provide accurate area and shape estimates, the hulls were projected independently using the Lambert azimuthal equal-area projection with the hull centroid used as the centre of the projection.

The shape of the geographical area sampled (predictor:  $Shape$ ) was estimated using the ellipse derived from a principal component analysis (PCA) of the projected convex hull with the centre of the ellipse located at the centroid of the hull. The ratio of the minor to major axis of the ellipse (or minor and major eigenvalues of the PCA) was used as a measure of shape, the closer this metric was to 1, the more circular the shape, the closer to 0, the more elongated the shape. In

addition to these species-level descriptors, we also estimated the pair-wise geographical distances between all populations of a species using the great-circle distance, calculated using the function `rdist.earth` available in the R library `spectralGP`. For each species these distances were then compiled into a distance matrix containing great-circle distances between all unique pair-wise combinations of populations.

### Environment

We selected a range of climatic and topographical variables to address the potential environmental basis of body size variation. Environmental data were extracted across a 50 km radius buffer of each population location. In order to minimize collinearity (and thus redundancy) among predictors an initially larger list of candidate variables was pared down to seven using a variance inflation factor  $\geq 10$  and partial correlation  $\geq 0.7$  as inclusion criteria. The single topographical predictor was elevation (m) which was based on the USGS GTOPO30 data set (<http://edc.usgs.gov/products/elevation/gtopo30/gtopo30.html>). All six climatic variables were derived from the CRU CL 2.0 average climatology database at a resolution of 10 arc minutes representing monthly averages for the period 1961–1990 (New *et al.* 2002). We averaged these values to generate one estimate of annual climate for each variable: average annual precipitation (mm), average diurnal temperature range ( $^{\circ}\text{C}$ ), range of average monthly temperatures ( $^{\circ}\text{C}$ ), range of average monthly precipitation (mm), average annual number of wet days (defined as  $> 0.1$  mm rain  $\text{day}^{-1}$ ), and average annual relative humidity (%). We then created distance matrices for each species based on the Euclidian distance between the vectors of environmental variables for all the unique pair-wise combinations of populations. The variables were standardized for each species to unit variance and mean zero before generating distance matrices.

### Analysis

We performed a two-step analysis. In a first, species-level analysis we sought to understand the differences in body mass variation across species as predicted by  $Clade$ ,  $Mass$ ,  $Area$ ,  $Shape$  and  $N$  using general linear models. All continuous predictors were  $\log_{10}$ -transformed before analysis. We determined the most parsimonious combination of predictors based on an exploratory analysis using an information-theoretic criterion (Burnham & Anderson 2002) and ranked models based on Akaike information criteria (AIC) scores and weights with a second-order correction for small sample sizes (AIC<sub>c</sub>). Because of the lack of *a priori* knowledge, we selected models for our assessment based on a sequential process starting with single predictor models followed by more complex models. The unconditional or intercept-only

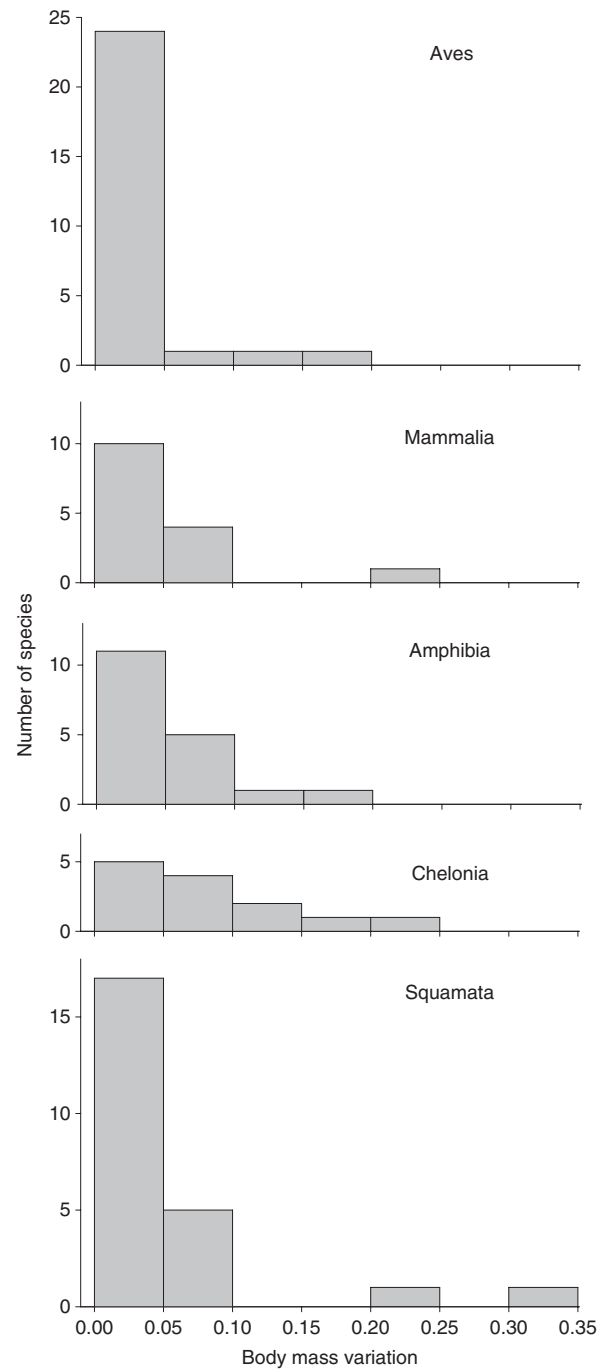
model and the saturated model were also included for reference. Once the most supported model was selected, we used linear contrasts to examine differences between clades. *P*-values for paired contrasts were adjusted using the Holm (1979) step down method to control the family-wise Type I error rate. To provide a preliminary quantification of phylogenetic signals we compiled a composite tetrapod phylogeny for the species in the data set and estimated the degree of phylogenetic dependence in the model residuals using a maximum-likelihood approach (Freckleton *et al.* 2002; Jetz *et al.* 2008). More details and results of this analysis are given in Appendix S1, Fig. S2 and Table S2.

In a second, population-level analysis we then used Mantel tests and partial Mantel tests to assess the concordance of differences in body mass among populations with geographical and environmental distances (Legendre & Legendre 1998). Mantel tests estimate the correlation between entries in two distance or dissimilarity matrices, and partial Mantel tests estimate the partial correlation conditioned on a third distance matrix. In this case, we examined the correlation between matrices of body mass differences and environmental distances and between matrices of body mass differences and geographical distances. We also examined, using partial Mantel tests, the same correlations with geographical distance and environmental distance used as conditional matrices respectively. The Mantel tests were applied to each species independently based on all the unique pair-wise combinations of populations for that species. We limited the assessment to species that had > 5 populations to allow for more complete representations of geographical and environmental gradients for each species. A total of 1332 populations across 67 species were considered in the assessment. Mantel tests were conducted using the R library *vegan* and the function *mantel*. The significance of Mantel test statistics was evaluated using permutation tests with 1000 permutations.

To examine differences in the distribution of Mantel test statistics within and among clades and between thermal regulation regimes, we used a variety of permutation procedures developed by Wilcox (2005) and implemented in R. To assess differences in average test values, we used the bootstrap-*t* method with 10% trimmed means and 9999 bootstrap samples. We used the function *tlwaybt* to examine multiple groups and for pair-wise comparisons the function *linconb*, which retained a simultaneous probability coverage of 0.05.

## RESULTS

The 98 species of terrestrial vertebrates differ markedly in their levels of cross-population variation in body mass (Fig. 1), with some species reaching a variation in body mass



**Figure 1** Population body mass variation for five clades of vertebrates estimated from the standard deviation of  $\log_{10}$  body mass. Based on 1447 populations across 98 species.

of 0.35 units standard deviations (of  $\log_{10}$  body mass), e.g. as in the Western Rattlesnake (*Crotalus viridis*, range of mean weights: 44.1 g–457.8 g,  $n = 7$  populations). In contrast, in many species, such as the Plains Pocket Mouse (*Perognathus flavescens*, 6.6 g–7.3 g,  $n = 14$ ) or the Downy Woodpecker

(*Picoides pubescens*, 25.5–27.2 g,  $n = 48$ ), body mass variation is close to undetectable and below 0.01 SD.

Overall we find that in all five clades most species show relatively low intraspecific variation, resulting in right-skewed frequency distributions of body mass variability (Fig. 1). With a median variation of just 0.016 and 0.019, birds and mammals are most constrained in their body size variation. In contrast, amphibians and turtles have highly variable body sizes compared to the other clades. Based on the saturated model containing all potential predictors, *Clade* membership is by far the strongest single predictor. But some of the variation is alternatively explained by species body *Mass*: large-bodied species tend to exhibit relatively greater within-species body mass variation (Table 1). Other single predictors, such as the *Area* or *Shape* over which populations were sampled and count of populations ( $N$ ), are not significant.

Given the potentially strong collinearity among these potential predictors (e.g. mammals are larger-bodied than squamates) we use a model averaging approach to identify the most parsimonious model. We use the variable *Clade* to represent the phylogenetic signal in this analysis (for

**Table 1** Intraspecific body mass variation predicted by effects of clade membership (*Clade*), number of populations ( $N$ ), species mean body mass (*Mass*), extent and shape of area covered by sampled populations (*Area*, *Shape*). Estimates for the levels within *Clade* are based on linear contrasts. The best-fit model is the most supported model according to  $AIC_c$  scores (Table 3). Significance levels are coded as follows: ●  $P < 0.1$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

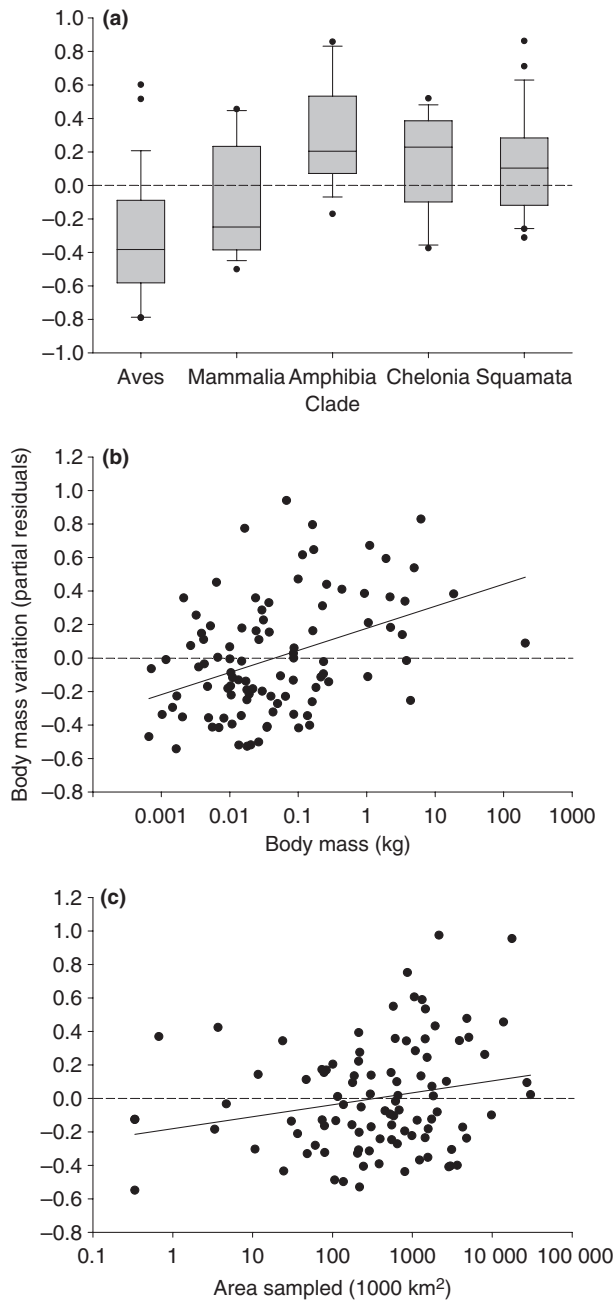
Predictor	<i>b</i>	<i>t</i>	<i>P</i>
Single predictors			
<i>Clade</i>			
Amphibia	0.16	2.03	*
Aves	-0.26	-4.10	***
Chelonia	0.26	2.99	**
Mammalia	-0.07	-0.76	
Squamata	0.07	1.01	
$N$	-0.14	-1.41	
<i>Mass</i>	0.08	2.08	*
<i>Area</i>	-0.01	-0.33	
<i>Shape</i>	-0.08	-0.51	
Best-fit model:			
<i>Mass + Area + Clade</i>			
<i>Mass</i>	0.13	3.02	**
<i>Area</i>	0.07	1.85	●
<i>Clade</i>			
Amphibia	0.26	2.98	**
Aves	-0.35	-4.89	***
Chelonia	0.22	2.14	*
Mammalia	-0.11	-1.25	
Squamata	0.10	1.42	

additional phylogenetic analyses see Appendix S1 and Table S2). Across all 16 linear model combinations of the five candidate predictors (interactions among them were not significant) three most-supported models emerge with  $\Delta AIC_c < 3.0$  (Table 2). The best-fit model has an Akaike weight of 0.50 and contains predictors from the other two and we therefore select it for further analysis (Fig. 2). This combined model confirms the effects already found for *Clade* and *Mass*, and additionally indicates a small positive effect of *Area* of sampled populations. Thus, even when controlling for among-species and among-clade differences in *Mass* (Fig. 2b) and *Area* (Fig. 2c), variability in body mass still differs substantially among the five clades (Fig. 2;  $F_{4,91} = 8.30$ ,  $P \leq 0.001$ ). The three indeterminately growing clades (turtles, squamates and amphibians) combined have greater variability in body mass on average when compared with the two determinant growing clades (mammals, birds;  $F_{1,94} = 23.51$ ,  $P \leq 0.001$ ). More specifically, amphibians have on average greater variability in body mass than birds ( $P \leq 0.001$ ) and mammals ( $P = 0.023$ ). Birds have on average lower variability than turtles ( $P = 0.006$ ) and squamatas ( $P \leq 0.001$ ).

We proceed to further investigate the causes of intra-specific body mass variation by evaluating population level effects. After finding a weak positive signal of *Area* over which populations are spread, we are interested in the

**Table 2** A comparison of 13 linear models examining differences in body mass variability among five clades (*Clade*) of vertebrates and different combinations of four covariates: average body mass across populations (*Mass*), area sampled (*Area*), general shape of the area sampled (*Shape*) and number of populations sampled ( $N$ ). All covariates were measured at the species level. Models are presented sequentially based on  $AIC_c$  scores

Model	d.f.	Adj. $R^2$	$AIC_c$	Delta $AIC_c$ ( $\Delta$ )	Akaike weight ( $w_i$ )
<i>Clade + Mass + Area</i>	7	0.36	69.89	0.00	0.50
<i>Clade + Mass</i>	6	0.25	71.17	1.28	0.26
<i>Clade + Mass + Area + N</i>	8	0.32	72.18	2.29	0.16
<i>Clade + Mass + Area + N + Shape</i>	9	0.26	74.61	4.72	0.05
<i>Clade + Area</i>	6	0.25	76.94	7.05	0.01
<i>Clade</i>	5	0.21	79.44	9.55	0.00
<i>Clade + N</i>	6	0.22	80.73	10.84	0.00
<i>Clade + Shape</i>	6	0.17	81.17	11.28	0.00
<i>Mass</i>	2	0.03	92.00	22.11	0.00
<i>Null</i>	1	0.00	94.23	24.34	0.00
$N$	2	0.01	94.30	24.41	0.00
<i>Shape</i>	2	0.00	94.91	25.02	0.00
<i>Area</i>	2	0.00	96.20	26.31	0.00



**Figure 2** Partial residuals of population body mass variation. The SD values were  $\log_{10}$ -transformed before analysis. The partial residuals are derived from a linear model examining variability in body mass among (a) five clades conditioned on the effect of two covariates, (b) average body mass and (c) area sampled.

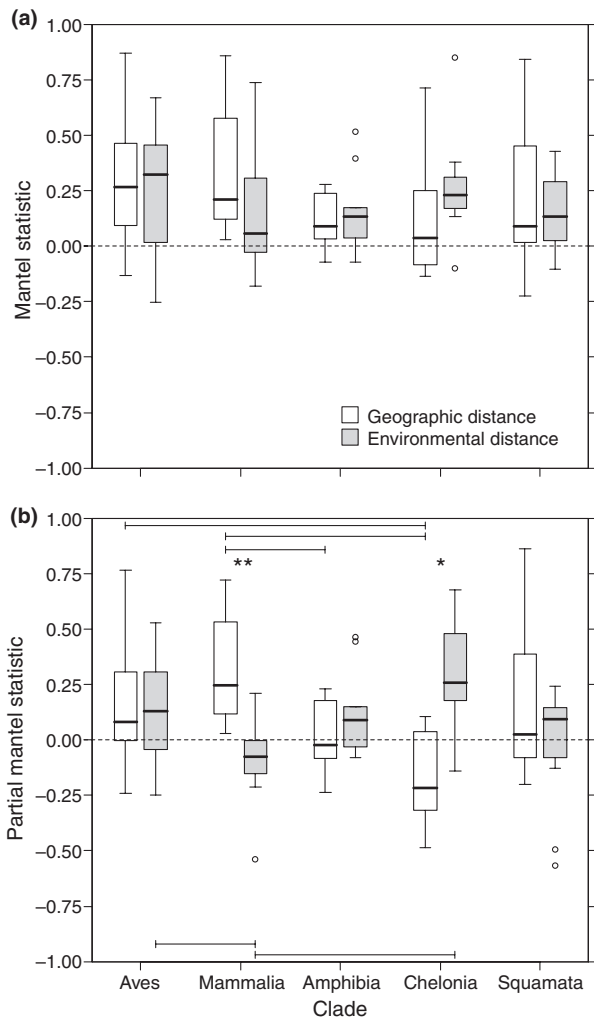
importance of geographical distance between populations vs. differences in environmental conditions as factors leading to the observed variation in body mass. Using pairwise within-species comparisons and Mantel tests for the 67 species with sufficient data, we find support for both

**Table 3** Summary of Mantel tests and partial Mantel tests applied to 1332 populations in five clades ( $N = 67$  species; only species with  $> 5$  populations were included). Mantel tests were used to examine the correlation between differences in body mass with geographical (Geo) and environmental (Env) distance, and the partial Mantel test was used to examine the correlation between differences in body mass conditional on the opposing factor, i.e. geographical distance controlled for environmental distance (Geo | Env Distance) and vice versa (Env | Geo Distance). For each clade the count of species with positive and negative trends is given (in brackets the count in which those trends were significant at  $P < 0.05$ ). For additional trends, see Fig. 3

	Geo <i>N</i>	Env Distance	Geo   Env Distance	Env   Geo Distance
<b>Positive</b>				
Aves	27 (17)	21 (17)	20 (14)	19 (15)
Mammalia	11 (5)	8 (5)	11 (5)	2 (2)
Amphibia	10 (1)	9 (1)	4 (1)	6 (0)
Chelonia	7 (3)	6 (3)	2 (1)	6 (3)
Squamata	12 (3)	9 (2)	7 (3)	8 (1)
<b>Total</b>	<b>67 (29)</b>	<b>53 (28)</b>	<b>44 (24)</b>	<b>41 (21)</b>
<b>Negative</b>				
Aves	27 (0)	6 (0)	7 (3)	8 (2)
Mammalia	11 (0)	3 (0)	0 (0)	9 (3)
Amphibia	10 (0)	1 (0)	6 (0)	4 (1)
Chelonia	7 (0)	1 (0)	5 (2)	1 (0)
Squamata	12 (0)	3 (1)	5 (0)	4 (2)
<b>Total</b>	<b>67 (0)</b>	<b>14 (1)</b>	<b>23 (5)</b>	<b>26 (8)</b>

(Table 3, Fig. 3a): in 43% of species body mass differences between populations have a significantly ( $P \leq 0.05$ ) positive correlation with geographical distance, and in 42% with environmental distance. In almost all cases of nonsignificant trends, the associations with either predictor are positive (Table 3). Within no clade is environment or geographical distance the stronger predictor of body mass differences ( $P = n.s.$  in both). Finally, no significant differences in associations between single clades emerge, but when considered jointly the effect of geographical distance is on average greater in determinate growers than in indeterminate growers ( $P = 0.043$ ).

Partial Mantel tests allow us to separate the independent effects of distance and environment. When controlling for environmental differences, the cross-population body mass differences of 36% of the species retain a significantly positive correlation with geographical distance (Table 3, Fig. 3b); 31% continue to show a positive association with environmental differences when controlled for geographical distance. But strong differences within and among clades continue to exist. Within mammals the independent effect of geographical distance exceeds that of environmental distance, whereas within turtles the opposite is the case



**Figure 3** Distribution of (a) Mantel test statistics and (b) partial Mantel test statistics for 67 species in five clades. For this analysis, only species with data for > 5 populations were considered, resulting in a total of 1332 populations. Mantel tests were used to examine the correlation between differences in body mass with geographical and environmental distances. Partial Mantel tests were used to examine the correlation between differences in body mass and geographical distance conditional on environmental distance and the correlation between differences in body mass and environmental distance conditional on geographical distance. Significant differences within the same measure and between clades are indicated by horizontal lines, between measures within the same clades with asterisks. There were no significant differences in (a). For the partial Mantel tests (b), clades connected with lines differ ( $P < 0.1$ ) based on the correlation between body mass and geographical (top) and environmental distances (bottom). A single asterisk indicates  $P < 0.1$  and a double  $P < 0.05$ . For further details, see Table 3.

(Fig. 3b). Across clades, mammals exceed turtles and amphibians in the independent importance of geographical distance, and the same applies to birds when contrasted with

turtles. The independent importance of environmental distance is greater with birds and turtles when contrasted with mammals.

The larger effect we observed of geographical distance alone on determinate growers (mammals and birds combined) compared with indeterminate growers (above, Fig. 3a) is confirmed when the association was conditioned on environmental distance ( $P = 0.010$ ; Fig. 3b). There was no such difference, absolute or conditioned, for the effect of environmental distance ( $P = \text{n.s.}$  in both).

## DISCUSSION

Our investigation found substantial interspecific differences in cross-population phenotypic variability. This finding has consequences for our understanding of the proneness of different taxa to exhibit population divergence, for the putative mechanisms giving rise to intraspecific eco-geographical trends, and for the usefulness of population measurements for characterizing species. Our results confirm three of our five original predictions, specifically those regarding the expected effects of growth type, body size and cross-clade differences. Our prediction regarding geographical range size was confirmed only in the model accounting for clade membership, but not when controlling for within-clade phylogenetic structure. Finally, not supported was our initial prediction about the effects of environmental heterogeneity which came out as smaller than anticipated.

Squamates, amphibians and to a lesser degree chelonians are known to have body sizes that are more plastic and on the whole less genetically controlled than birds and mammals (Rutledge *et al.* 1973; Ellison *et al.* 1993; Freckleton *et al.* 2003), and to exhibit growth asymptotes that can differ strongly between habitats (Berven 1982; Sinervo & Adolph 1994; Ashton & Feldman 2003). Further, in many cases growth may even be truly indeterminate and can result in lifelong size increases or even shrinking in response to environmental conditions (Sebens 1987; Wikelski & Thom 2000). As expected, these three clades show the greatest body mass variation across populations, which supports in this group the potential importance of direct and local effects (such as food availability and temperature on growth rates, competition and predation) on locally possible or optimal body sizes. We show that this higher phenotypic variability exists above and beyond the strong clade differences in body size and geographical range size. However, we note that the very small number of major evolutionary shifts in growth type that the clades in our study represent puts limits on the interpretation.

Species of widely different body mass (and thus metabolic costs of locomotion) and ecology interact with their surrounding landscape at different grains, and experience

environment and spatial distance very differently. In terrestrial vertebrates dispersal abilities are higher for flyers and greater for large-bodied species (Alexander 1998). Between mammals and birds, the latter exhibit stronger effects of environmental heterogeneity controlled for spatial distance – potentially a consequence of their much greater mobility and the resulting connectivity of populations which would counteract differentiation. The overall strong positive effect of body mass on phenotypic variability could be interpreted as a signal of smaller levels of regional gene flow in large-bodied species. Individual encounter frequencies (and thus opportunities for genetic exchange) are expected to decrease with increasing body size, at least at landscape scales, driven by the decrease in population density towards larger body sizes that is not compensated by a relatively small increase in mobility with body mass (Jetz *et al.* 2004). At this point, empirical estimates of gene flow to support this notion are not available across multiple species. But given the dramatically growing phylogeographical knowledge, in the near future a specific test of this hypothesis and its presumed connections may be possible.

There may be alternative explanations for the positive effect of body mass on its variability. There is good evidence that within populations phenotypic heterogeneity can vary predictably, e.g. (controversially) in relation to a population's niche breadth in a given location (Van Valen 1965; Soule & Stewart 1970; Bolnick *et al.* 2007). Assuming that large-bodied species have broader niches, they should therefore show greater variability. But this hypothesis is focused on within-population variability, and cannot straightforwardly be extended to cross-population patterns. A second alternative explanation is the idea that differences in cross-population phenotypic variability between species may arise for reasons to do with species' biology rather than the geography of populations. Variability in body mass may show a trend with the size of organisms due to consistent changes in the composition of organisms. The relative contribution of different organ systems to total body mass may become more variable towards larger body masses, as some components may be fixed in size and others vary (Hallgrímsson & Maiorana 2000). Again, this variability should express itself more strongly within than across populations. Finally, potential biases due to measurement errors are unlikely to explain our result: in relative terms measurement errors should be largest in small-bodied species, which is opposite to the trend we observe.

As predicted, we also find that for the three clades with purported weaker genetic control of body size, spatial distance between populations has a lesser effect on body size differences than in mammals and birds. Across all clades, body mass divergence increases with increasing total area covered by populations. We illustrate that in many cases this increase in population differentiation can be explained

by higher environmental heterogeneity and larger geographical separation of populations. Avian and mammalian body sizes are under intense genotypic control (Ellison *et al.* 1993; Freckleton *et al.* 2003), and we find that for both combined body mass variability is higher in geographically more distant populations, above and beyond the environmental differences that separate them, than is the case for the other clades. Further, in one comparison (mammals and turtles) there is also greater relative importance of environmental rather than geographical distance in the clade with expectedly more plastic phenotypes.

However, a clear dominance of environmental heterogeneity and with it a signal of local adaptation as opposed to simple geographical distance in explaining body size differences did not emerge. This suggests a strong relative importance of non-adaptive causes of cross-population body mass variation, which clashes with a purported strong effect of environmental gradients on geographical patterns of body size variation, as e.g. expected under an intraspecific interpretation of Bergmann's rule (Mayr 1963; James 1970). The purpose of this study was to test joint environmental effects without highlighting single variables or supposing directionality, rather than e.g. to describe body size increases with latitude across populations. In our analysis we combined seven environmental predictors to maximize the ability to attribute environmental signal to population differentiation. Despite this, absolute environmental dissimilarity was on the whole not much better in differentiating population body masses than pure spatial distance. This finding casts doubt on the strength and relative importance of Bergmann-type effects on the geography of body masses, and instead emphasizes the importance of gene flow for the geographical variation and existence of clines in body size (Haldane 1948; Gould & Johnston 1972). In contrast to these findings, there is also evidence that gene flow alone can have relatively little impact on genetic and phenotypic variability. For instance, Endler (1973) showed experimentally and theoretically that intraspecific clinal variation evolves in response to selection via different environments, but that gene flow alone may play only a small role. Storz (2002) reported that additive genetic effects for body size divergence among populations of a fruit bat, *Cynopterus sphinx*, were significantly associated with both geographical and environmental distance even after partialling out the effects of neutral genetic divergence. In other words, species body size variability across populations appears to be strongly due to selection, with gene flow playing a less important role. At this point there is not enough genetic information readily available to jointly assess phenotypic and genetic variation across multiple populations and many species. But with the increased gathering of geo-referenced and joint morphological, phylogeographical data an integrative broad-scale ecological and evolutionary analysis into the



drivers of population phenotypic variability is an exciting prospect.

Species occurring over large geographical areas may not only encounter more environments but also a greater number of ecologically or morphologically similar species. Competitive interactions may be reduced and coexistence of similar populations be facilitated by species sorting or ecological character displacement (Brown & Wilson 1956; Hutchinson 1959; Grant 1972). Geographical population differentiation may further arise not only from spatial turnover in competitors but also from spatial turnover in prey (McNab 1971). Some of the spatial biotic turnover may be explained by the turnover in environmental conditions and thus captured in our analysis, but especially outside the tropics that association may be weak (Buckley & Jetz 2008). Most of all, co-occurrence with competitors and resulting population-level character divergence is expected to be a function of geographical distance (Davies *et al.* 2007). It follows that the relatively strong effect of geographical distance on body mass variability could alternatively be explained by the effects of competition (Meiri *et al.* 2007). Lacking information on potential competitors and prey across the 1447 populations in this study, we can only speculate about this idea. Further broad-scale yet detailed ecological information is needed to fully evaluate this hypothesis, but it appears a promising avenue.

In conclusion, we show that terrestrial vertebrates exhibit marked global variation in population body mass divergence. The differences between species are best explained by phenotypic plasticity, correlates of gene flow, and the geographical and environmental distance among populations. The understanding of patterns and adaptive vs. non-adaptive causes of the geographical phenotypic variation between species and at broad scales to date has been limited, but may be an exciting catalyst for conceptual integration. In the near future, increased ecological and phylogeographical data at broad scales and across many species hold the potential for critical additional tests and further synthesis.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Geographical locations of the 1447 populations considered in the analysis. A total of 98 vertebrate species from five vertebrate clades were represented.

**Figure S2** Composite tetrapod phylogeny used for phylogenetic analyses in this study. The tree was compiled from several sources.

**Table S1** Characteristics of the data across clades used in this study: count of species, and the number, area and body mass of populations sampled. Maximum and minimum values per species are given in parentheses.

**Table S2** Analysis of intraspecific body mass variation, controlled for phylogeny (cf. Table 1; for tree, see Fig. S2)

**Table S3** The best-fit model of intraspecific body mass variation, controlled for measurement type (cf. Tables 1 and 2).

**Table S4** Summary of Mantel tests and partial Mantel tests applied only to those species with direct body mass measurements (cf. Table 3), totalling 548 populations in five clades ( $N = 33$  species).

**Table S5** The 98 species of tetrapods considered in the analyses with the number of populations sampled ( $n$ ), the average of  $\log_{10}$  body mass (mean), the standard deviation of  $\log_{10}$  body mass (SD) and sources for the body mass information.

**Appendix S1** Phylogenetic analysis.

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