The evolution of reproductive strategies in turtles

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Abstract

The ~~recorded~~ variation in egg and clutch ~~traits~~ among amniotes has led to the idea that selection towards an "optimal egg strategy" is based on trade-offs. Here we ~~report results of analyses on~~ egg and clutch characteristics across all ~~large~~ clades of living turtles, including at least one representative of each extant turtle genus. ~~Our goals were to investigate~~ whether egg and clutch size follow the predictions of egg size theory, if there is convergence regarding reproductive strategies among turtles, and to identify factors that influence clutch and egg traits ~~in this amniote clade~~. We hypothesized that egg and clutch characteristics are influenced by a number of factors, including ecological constrains, and that reproductive traits in Testudines evolved independently several times in distantly-related clades. We find evidence for incomplete convergent evolution based on egg and clutch traits. Diet and climatic zone play important roles in the selection towards optimal reproductive strategies in different species.

Introduction

The macroevolutionary patterns in amniote reproduction (Battistella et al., 2019; Murray et al., 2020) can be investigated based on the diversity of traits in the egg and clutch (e.g., Kaplan and Salthe 1979; Deeming and Birchard, 2007; Jetz et al., 2008; Deeming and Ruta, 2014). The idea of an "optimal" correlation between egg and clutch size, based on trade-offs, and similar to K/r strategies, has led to several inconclusive discussions (Congdon and Gibbons 1987; Elgar and Heaphy 1989; Godfray et al., 1991; Kuchling, 1999; Li et al., 2017; Yu and Deng, 2020). Optimal egg/clutch size theory assumes that changes to the egg and clutch are driven by selection, resulting in adjustments for the largest possible production of offspring with the highest fitness, at the lowest resource cost to their progenitors (Brockelman, 1975; Congdon and Gibbons, 1987, Janzen and Warner, 2009).

Turtles ~~offer a rich subject of investigation~~ given the ecological diversity of the group. Studies focused on turtles have tested many correlations between egg size and both morphological and ecological traits in an effort to explain the variation among species (Elgar and Heaphy, 1989; Iverson, 1992; Iverson et al., 1993, 2019; Rowe, 1994; Rachmansah et al., 2020). Some authors have argued that the "optimum" egg size is determined by adult body size (Gibbons, 1982), pelvic aperture morphology (Congdon and Gibbons, 1987; Kuchling, 1999; Clark et al., 2001; Hofmeyr et al., 2005), environmental factors (Hofmeyr et al., 2005; Macip-Ríos et al., 2013) and/or physiology (Bowden et al., 2004). These hypotheses, largely based on studies of trait correlations (Gibbons, 1982), can be tested by methods that consider phylogeny, as they reduce the variance of estimated regressions (Rohle, 2006).

Available evidence supports the idea that large-bodied animals tend to produce larger clutches with relatively small and round eggs (Fig. 1A), while smaller species produce small clutches with relatively large and elongated eggs (Fig. 1B). Elgar and Heaphy (1989) proposed that spherical eggs are less susceptible to desiccation as the surface-volume ratio is smaller in comparison to elongated eggs. In contrast, Pritchard (1979) suggested that small species tend to produce bigger, elongated eggs because a small spherical egg would not be capable of producing a functional hatchling due to a lack of space, and that adult body size is a constraint for the egg width. Moll (1979) argued that spherical eggs occupy space more efficiently, thereby allowing for larger clutches.

Although general trends have been identified (e.g., Iverson et al., 2019; Rachmansah et al., 2020), a comprehensive analysis exploring egg and clutch characteristics across all genera of living turtles is still missing. We present analyses based on data from the literature for at least one representative of all extant turtle genera, in order to identify trends of their reproductive strategies and investigate potential factors that influence clutch and egg traits in this clade. We addressed the following questions: 1) Are reproductive traits (such as egg size, egg shape, and clutch size) correlated as predicted by egg size theory? 2) Are there “optimal” egg strategies on which distantly related species converge? 3) Do ecological factors influence egg/clutch characteristics? We hypothesized that egg and clutch characteristics follow the predictions of egg size theory and that they are influenced by several factors, including ecological and phylogenetic constraints. Furthermore, we hypothesize that reproductive traits in Testudines evolved independently several times across clades that are not directly related, which can be considered evidence of convergence on optimal reproductive strategies.

Materials and Methods

We collected morphological, ecological and reproductive data for at least one species of each turtle genus (Table 1; Supplemental Information, Appendix S1) using available literature (Supplemental Information, Appendix S2). The search was conducted following PRISMA (Moher et al., 2011) guidelines. All statistical and exploratory analyses were conducted in the R statistical environment (v.4.0.4) (R Core Team 2016; scripts and input files available in Supplemental Information, Appendix S3).

Exploratory analysis

In order to explore the distribution of reproductive parameters (egg width, egg length, adult body size, clutch size and number of clutches per year) commonly explored in previous works with smaller datasets (Elgar and Heaphy, 1989; Iverson, 1992; Iverson et al., 1993; Rowe, 1994), we performed a phylogenetic principal component analysis (pPCA) using the function *phyl.pca()* (package phytools; Revell, 2012) and the phylogeny proposed by Pereira et al. (2017). ~~We used the first two axes of the pPCA as source information to plot the tree in morphospace, which can be defined as the multidimensional distribution of an organism's phenotype (Lloyd, 2018). The incorporation of phylogenetic information (tree topology) provides not only information on phenotypes disparities, but also on the transformation from ancestral to derived conditions, leading to a phylomorphospace (Gerber, 2019).~~ We used the function *phylomorphospace()*, also from the phytools package (Ravell, 2012).

We used the *contMap()* function of the phytools package (Revell, 2012) to map the reproductive trait characters in turtles. This analysis was run three times, using respectively the following characters: 1) Egg size (ESI): egg length/carapace length; 2) Egg "shape" (ESH): egg length/egg width; and 3) Fecundity (FEC): maximum number of eggs in a clutch, times the mean number of clutches per year. ~~Although the function is useful for reconstructing ancestral characters, our primary goal was to use its visual capabilities for tree mapping to facilitate the visualization of state distribution on the tips~~. For that reason, we chose to use a more inclusive topology that allows a broader visualization of trait distribution. The tree was informally reconstructed in Mesquite v. 3.51 (Maddison & Maddison, 2019), with no information on branch lengths. We used the tree from Pereira et al. (2017) for the backbone of our tree and grafted other species based on other phylogenetic hypotheses: Pelomedusidae (Fritz et al., 2011); Podocnemididae

(Vargas-Ramirez et al., 2008; Guillon et al., 2012); Chelidae (Georges et al., 2002; Vargas-Ramirez et al., 2012; Le et al., 2013; Zhang et al., 2017); Geoemydidae (Le et al., 2007; Guillon et al., 2012; Pereira et al., 2017); Testudinidae (Pereira et al., 2017); Emydidae (Fritz et al., 2012; Pereira et al., 2017; Thomson et al., 2018); and Kinosternidae (Iverson et al., 2013).

Explanatory analysis

To test the hypothesis of convergence on reproductive traits of turtles, we ran a series of ~~evolutionary~~ model-fitting analysis ~~(process-based test)~~ using the mvMORPH R package (Clavel et al., 2015). We tested the fit of seven ~~different~~ multiple optimum Ornstein–Uhlenbeck ~~process~~ models (OUM, Table 1), using a single-regime Brownian motion model (BM1), a multiple mean Brownian motion model (BMM) and a simple Ornstein–Uhlenbeck process model (OU1) as null hypotheses (rejection of convergence). Each OUM represents a hypothesis of regimes with two or more convergent peaks caused by different selective pressures, represented as “optima” (θ, Hansen, 1997; Butler and King, 2004). To test the fitting of multiple regime models, we assigned ancestral traits to the trees by using the *make.simmap()* function from the phytools R package (Revell, 2012). For this analysis we also used the tree proposed by Pereira et al. (2017). Trait-assigned trees can be found in supplemental material (Figure 1; Supplemental Information, Appendix S4). Model fitness was evaluated using Akaike information criterion (AIC) values (Akaike, 1974).

Complementarily, we ran a MANOVA to test the significance of the correlations between the regimes of the selected model and the traits of eggs and clutches. We used the functions *mvgls()* and *manova.gls()* from the package mvMORPH (Clavel et al., 2015).

In order to estimate the relationship among reproductive traits and ecological factors considering the phylogenetic relationships among Testudines, we ran two different phylogenetic generalized least square (PGLS) models. In the first analysis, we used ESI (egg size) as the dependent variable to test how the different independent variables (climatic zone, diet and the log clutch mean, Table 2) predicted the size of the egg among species. We log-transformed the mean number of eggs per clutch (clutch mean) to avoid skewed distribution of the predictor. In the second analysis, we used the maximum number of eggs laid in one clutch times the mean number of clutches per year as a proxy for fecundity, to test how the selected independent variables (climatic zone, diet and egg size, Table 3) predicted the fecundity of turtle species. We log-transformed the fecundity variable in order to achieve homoscedasticity and normality of residuals~~ in the model~~.

In both analyses we used the tree from Pereira et al. (2017) and included ~~a total of~~ 160 species (Supplemental Information, Appendix S3). We removed *Pyxis arachnoides* and *Amyda cartilagina* from the analyses to guarantee the homoscedasticity and normality of the model, as they presented high residual values, ~~worsening the model fit~~. Habitat and zoogeographic zones were not used due to the lack of cross-observations with other variables and collinearity with the variable climatic zone. We used maximum likelihood and Brownian motion correlation to fit both PGLS, using the function *gls()* of the package nmle (Pinheiro and Bates, 2020).

For model inference we used a Full-Null Model Comparison approach. We calculated the percentage of variance on the dependent variable that is explained by the model based on the likelihood of observing the data (R2 lik), and tested for each predictor's significance and confidence intervals.

Results

~~Exploratory analysis~~

~~The contMap analysis (Fig. 2A, B and C) allows easy visualization of character distribution throughout the tips of the phylogenetic tree. By comparing plotted trees, it is possible to correlate small and round eggs to species that produce bigger clutches. Such traits evolved independently in several distant-related families (e.g., Podocnemididae, Cheloniidae, Dermochelyidae, Chelydridae) or only in larger-bodied representatives of certain families (e.g., Testudinidae, Trionychidae).~~

~~The morphospace analysis (Fig. 2D) provides a holistic view of trait distribution in the phylogeny.~~ Most species occupy two ~~main~~ regions of the multivariate morphospace. Species distributed towards higher values of PC1 have larger body size and tend to produce bigger clutches with relatively smaller eggs compared to species distributed on the opposite quadrant of the morphospace (follow the R script on supplementary material, Appendix S3, to add tip names to the plot).

Explanatory analysis

The model-fitting analysis recovered diet (OUM) as the best fitted model (Table 1) with over 96% Akaike weight (AICw). The only other model with AICw values over 0.01 was habitat (OUM). The MANOVA test among diet regimes and reproductive traits was non-significant (p=0.371).

All the independent variables (climatic zone, diet and clutch mean) were significant in predicting egg size in turtle species in the first PGLS analysis (Table 2; Fig. 3). The model explained (R2 lik) 38% of the variation in the egg size. Diet and the size of the egg predicted fecundity in turtles, but climatic zone was not significant (Table 3; Fig. 4). The model explained (R2 lik) 33% of the variation in fecundity.

Discussion

The evolutionary history of turtles is marked by a complex pattern of character evolution regarding their reproductive strategies (e.g., changes in egg size, egg shape and clutch size). Our analyses support the interpretation of repeated changes in these characters over the evolutionary history of different turtle groups. Furthermore, the hypothesis that large-bodied turtles tend to produce larger clutches with comparatively smaller and rounder eggs, while small-bodied species produce small clutches with larger and more elongated eggs seems to be supported by general patterns described in both the analyses here as well as those in previous literature (Elgar and Heaphy, 1989; Iverson, 1992; Iverson et al., 1993, 2019; Rowe, 1994; Rachmansah et al., 2020).

Our exploratory analyses show results consistent with the egg size theory. In the contMap analyses, traits (evidenced by different colors) tend to interact with each other, suggesting a correlation between the tested characters. This pattern is partially recovered in the phylomorphospace. Two main clusters can be separated based on their distribution in different PC regions, correlated with the species' reproductive characters, although subjected to personal interpretation. In any case, based on the distribution of characters along the tree, we can assert that these patterns evolved independently and recurrently along the diversification of turtles. This shows that during their evolutionary history, turtles explored different reproductive strategies, with several instances of convergent evolution.

However, correlation does not imply causation and the interpretation of observed patterns as an example of evolutionary convergence is not straightforward ((Kluge 2005; Stayton, 2015b). To be able to make inferences about evolutionary patterns, we used quantitative measures and falsified our hypotheses with null models (Popper, 1982; Stayton, 2015b). Among all evolutionary models tested, the distribution of groups categorized according to diet (herbivores, omnivores and carnivores) showed the best fit, suggesting that there are three reproductive strategies to which turtles converge. This also supports the hypothesis that ecological factors, in this case diet regimens, have an important role in the tested reproductive traits, although the MANOVA results were not significant.

Concordantly with the model-fitting analysis, diet was the only factor to partially explain the correlations in both PGLS analyses. Herbivores have relatively smaller eggs while carnivores have relatively higher fecundity. Climatic zone was also an influential factor on egg and clutch size. Tropical species have smaller eggs and a higher mean number of eggs per clutch compared to species from temperate areas. These results might be influenced by the broad availability of resources in tropical areas, which enables larger-bodied taxa that can produce more eggs (Rachmansah et al., 2020). It might also play a role in egg nutrition (Craven et al., 2008) and in favoring earlier hatching, as tropical areas have higher mean temperatures throughout the year, which increases metabolism in embryos (Spencer and Janzen, 2011).

Aside from the importance of ecological factors in egg and clutch characteristics, the PGLS analyses also support our exploratory analyses. There is a negative correlation between relative egg size and fecundity, demonstrating that reproductive traits are correlated as predicted by egg size theory.

Based on these results, we advocate that there are major trends in reproductive strategies to which turtles converge. These trends seem to be somewhat relaxed as all reproductive characters used are continuous, without any clear breaks in patterns, and most species show average values clustered together in the morphospace analysis. The lack of significance in the MANOVA results after the evolutionary model-fitting analysis, together with the lack of obvious converging subgroups in the morphospace analysis can be considered as indications of a case of incomplete convergence. Such cases have been reported before in other vertebrate clades (such as in mammals. e.g., Meloro et al., 2015; Grossnickle et al., 2020).

Clades that represent cases of incomplete convergence can be described as being similar – or more similar than their ancestors – but presenting distinctive phenotypes (Herrel et al., 2004; 2015a; Losos, 2011). In addition to the characters tested in the present work, other factors might play important roles in egg and clutch strategies of turtles and could be responsible for shaping the patterns found in our analyses. Adaptations within specific niches are worth mentioning and should be taken into account when interpreting this complex scenario (see Kluge 2005 and Losos 2011 for a review of the role of convergent evolution in inferring adaptations).

Little is known about many of the aspects that influence the reproductive characteristics within Testudines, which include specific environmental pressures (as suggested by Hofmeyr et al., 2005 for *Homophus signatus*; and by Hedrick et al., 2018 for *Chelydra serpentina*, the last case within annual changes over the same population), patterns of reproductive allocation within and among species (Wilkinson and Gibbons, 2005), conflicts in parent-offspring size (Janzen and Warner, 2009), anti-predatory strategies (Santos et al., 2016), maternal effects and parental care (Hughes and Brooks, 2006; Warner et al., 2010).

As Nussbaum (1987: 38) stated: “The safe harbor hypothesis includes the suggestion that parental care causes the embryonic stage to be the safest harbor, and, therefore, egg size will increase in populations with parental care to decrease the duration of subsequent, higher risk stages”. Testudinidae is the turtle clade with the largest number of species known to care for their eggs (Agha et al., 2013). Although still an uncommon behavior within this clade, it makes the safe harbor hypothesis a possible explanation for the comparatively larger eggs and, consequently, smaller clutches in most species of this clade.

Although other turtle clades have historically been considered to lack any form of parental care, there is now evidence to the contrary (Ferrara et al., 2013). The Arrau turtle (*Podocnemis expansa*) is the biggest South American freshwater turtle, and produces many small round eggs in a clutch. In this case, the only described parental care behavior starts after the eggs hatch, providing the safe harbor hypothesis with only weak explanatory power. Other factors probably have a bigger influence in this case, such as the proposition that round eggs suffer less from desiccation (Elgar and Heaphy, 1989; Hofmeyr et al., 2005).

As noticed by Elgar and Heaphy (1989: 137), “Terrestrial species lay fewer and larger eggs for their size than freshwater or marine species, but this association is statistically confounded by the fact that chelonian families form ecological groups”. The convergent distribution of reproductive traits and the different modifications of these traits across families that occupy unique niches—such as Testudinidae that live on land and Cheloniidae/Dermochelyidae that live in the ocean—could be considered evidence for the adaptation of specific clades to an “optimal” reproductive strategy in a specific environment or under a specific constraint.

The fact that the evolution of these strategies is correlated with the colonization of new environments provides strong support for a heuristic assumption of adaptive value (Kluge 2005; Losos, 2011). At the same time, asserting the adaptive value of some of these traits can be difficult (see Kluge, 2005), and the correlation between specific traits and families that form ecological groups prevents the postulation of statistically supported tests, which makes hypotheses based on niche adaptations greatly speculative (Popper, 1982; Stayton, 2015b).

Conclusions

We conclude that reproductive traits in Testudines evolved independently several times across non-directly related clades. This can be considered evidence of convergence and an endorsement of the existence of adaptive evolution and constraints in reproductive biology, frequently referred to as “optimum” reproductive constraints. Many different aspects may play important roles in the selection of specific “optimum” reproductive strategies in Testudines, preventing species from fully reaching convergence.

Egg and clutch characteristics follow the trade-offs predicted by egg size theory and are influenced by ecological factors. Both diet and climatic zone play important roles in the distribution of reproductive characteristics among turtle clades.

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Figure 1

Egg and clutch strategies

Examples of different strategies are shown: nest of the giant Amazon river turtle (*Podocnemis expansa*) with many small round eggs (A); small clutch with big and elongated eggs of the South American wood turtle (*Rhinoclemmys punctularia*) (B). The adult? carapace length of these two species reaches over one meter and 25cm long, respectively.

Figure 2

Exploratory analyses of the phylogenetic distribution of Testudines reproductive traits

ContMap analyses (A-C) allow the visualization of character distribution along the three. A) Egg size (ESI); B) Fecundity (FEC); and C) Egg shape (ESH). Colors represent size measurements, with red, orange and yellow indicating small egg size, low fecundity and rounder eggs (starting from 0.025 cm, 1 unit and 0.025 cm, respectively) and blue representing large eggs, high fecundity and elongated eggs (with maximum values of 0.395 cm, 168 units and 2.611, respectively). See bars on the bottom of each tree for color reference. D) Phylomorphospace based on the first two PCs of turtle traits PCA. Each color represents one different family, following the combinations available on figure 2C.

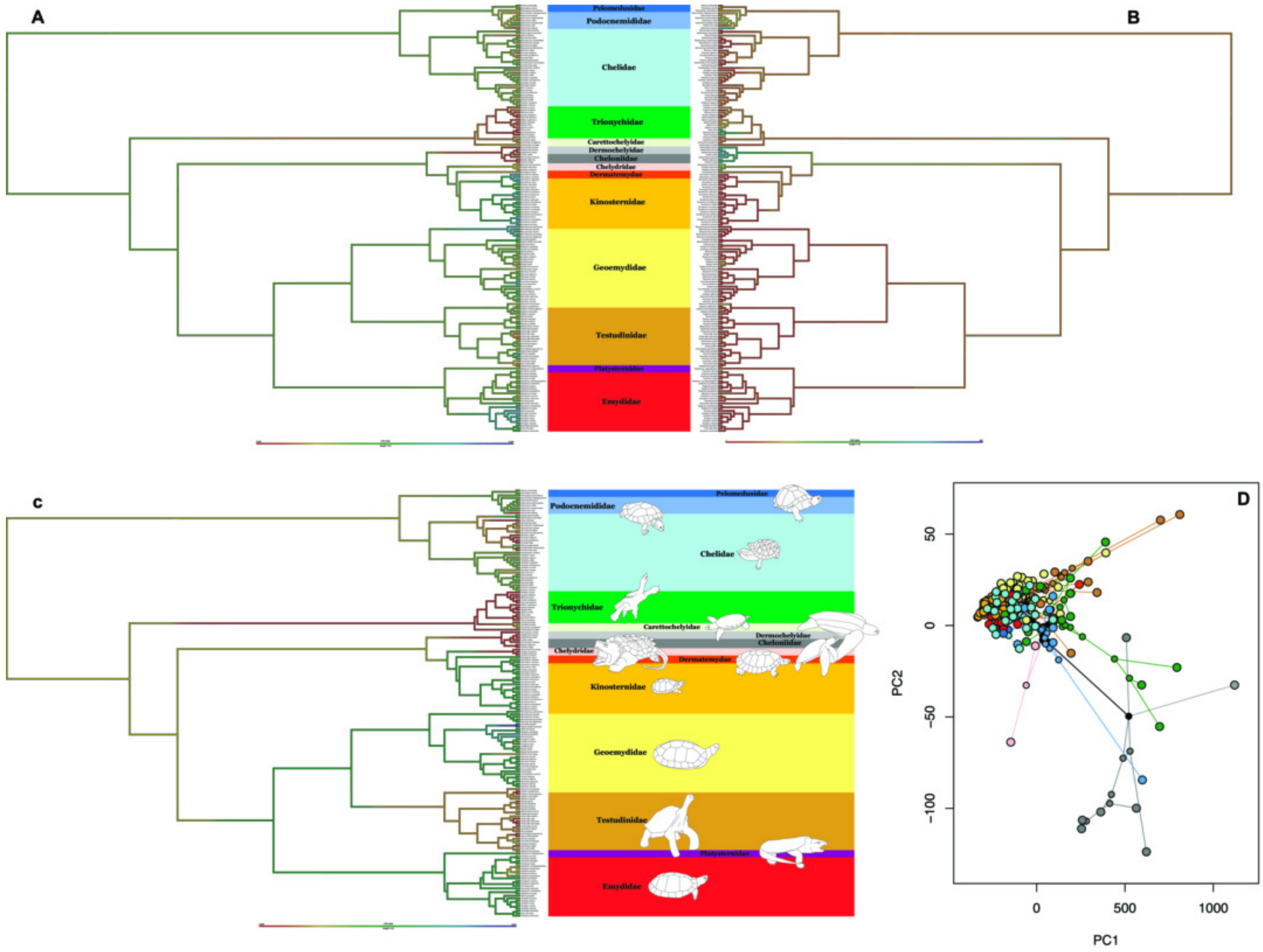
A
B
Pelomedusidae
Podocnemididae
Chelidae
Trionychidae
Carettochelyidae
Dermochelyidae
Cheloniidae
Chelydridae
Dermatemydidae
Kinosternidae
Geoemydidae
Testudinidae
Platysternidae
Emydidae
c
D
50
0
−50
−100
PC2
0
500
1000
PC1

Figure 3

Phylogenetic Generalized Least Squares model of variables predicting egg size in turtles

The model predicts the relationship of relative egg size (egg length/carapace length) to log mean clutch size (mean number of eggs laid per clutch) for turtle species that occupy different climatic zones and have different diet types

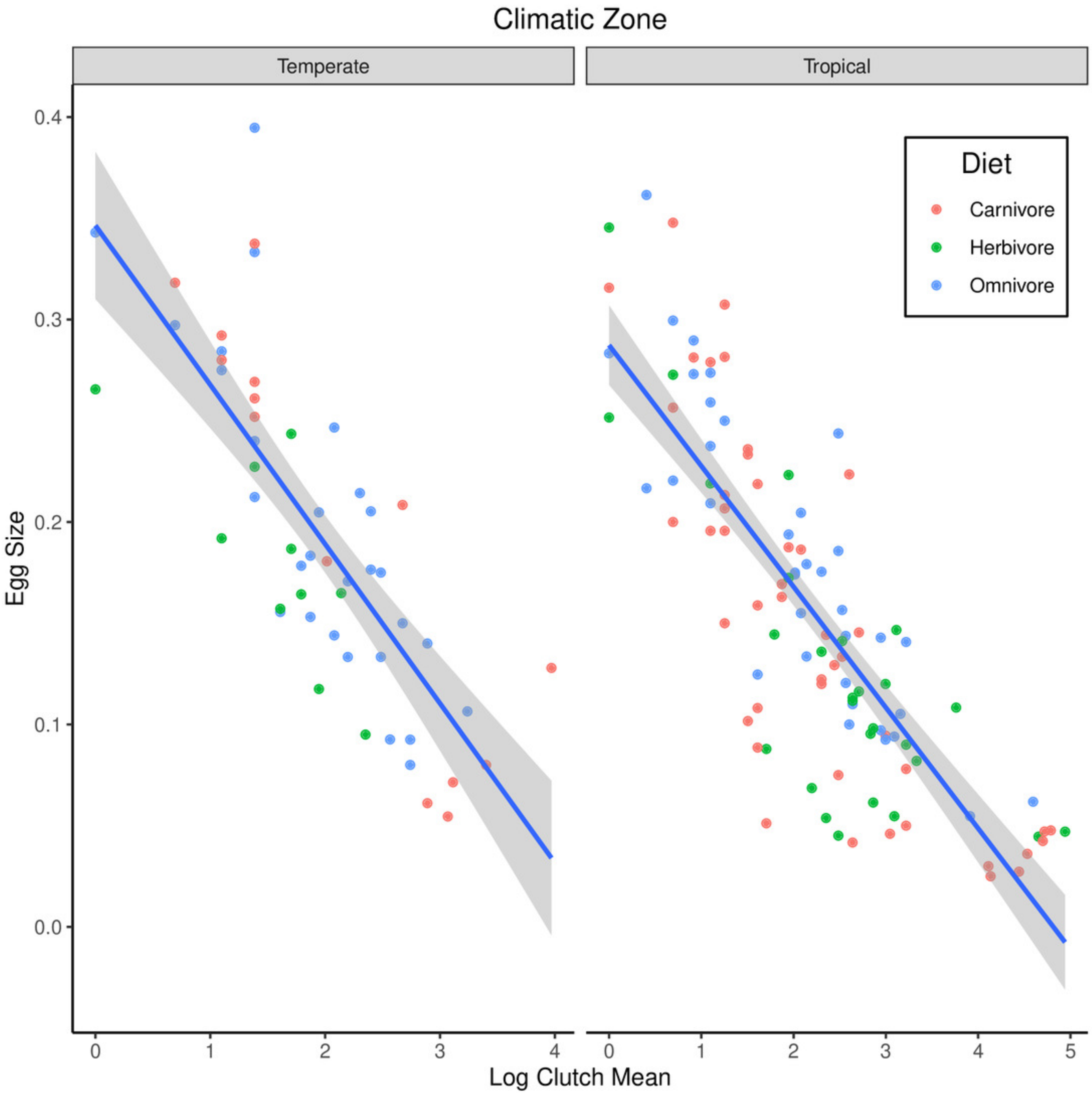
Climatic Zone
Temperate
Tropical
Diet
Carnivore
Herbivore
Omnivore
Egg Size
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
0
1
2
3
4
5
Log Clutch Mean

Figure 4

Phylogenetic Generalized Least Squares model of variables predicting fecundity in turtles

The model predicts the relationship of log fecundity (maximum number of eggs laid per clutch times the mean number of eggs laid per clutch) to relative egg size (egg length/carapace length) for turtle species that have different diet types. We do not report climatic zone in this figure due to the non-significance of the predictor

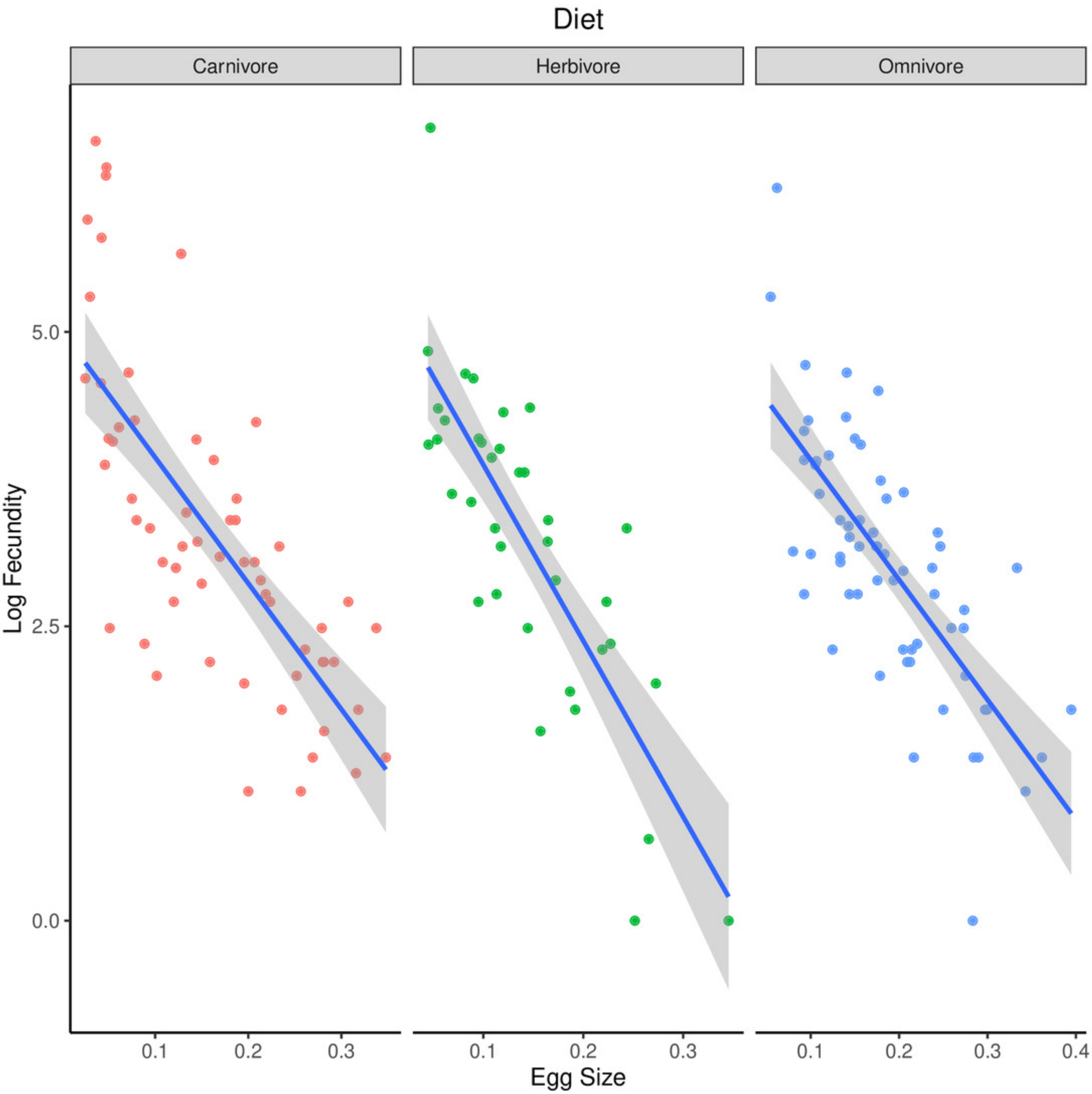
Diet
Carnivore
Herbivore
Omnivore
Log Fecundity
5.0
2.5
0.0
0.1
0.2
0.3
0.1
0.2
0.3
0.1
0.2
0.3
0.4
Egg Size

Table 1(on next page)

Fitting scores of multivariate evolutionary models to egg and clutch data

Model details and respective ancestral state tree reconstructions are available on supplementary information (Figure S1). Diet (OUM, in bold) is the best-fitted model. BM1, simple Brownian motion model. BMM, Multiple mean Brownian motion model. OU1, simple Ornstein–Uhlenbeck process model. OUM, Multiple optimum Ornstein–Uhlenbeck process models. LogLik, The log-likelihood of the optimal model. AIC, Akaike Information Criterion for the optimal model. AICc, Sample size-corrected AIC. Diff, AIC difference with the best fit model. Wi, absolute Akaike weights. AICw, relative Akaike weights

System	θ	Model	LogLik	AIC	AICc	diff	wi	AICw
Control	1	BM1	-335.662	689	689.6996	192	0	0
Control	1	BMM	-297.689	649	652.6572	152	0	0
Control	1	OU1	-236.065	508	509.5853	10.8	0.00455	0.004388
Clima	2	OUM	-233.335	509	510.649	11.3	0.00347	0.003349
Diet	**3**	**OUM**	**-224.672**	**497**	**499.9301**	**0**	**1**	**0.964863**
Habitat	3	OUM	-228.372	505	507.3297	7.4	0.02473	0.023859
Zoogeography	7	OUM	-220.528	513	518.95	15.7	0.00039	0.000374
Body size	2	OUM	-235.905	514	515.7892	16.5	0.00027	0.000256
Body size	3	OUM	-233.303	515	517.1922	17.3	0.00018	0.000172
Body size	4	OUM	-227.537	509	512.3532	11.7	0.00284	0.002738

1

Table 2(on next page)

Phylogenetic Generalized Least Squares scores of variables predicting egg size in turtles

Climatic zone, diet and clutch mean predict the size of the egg in turtle species. SE, standard errors. CI, confidence intervals

Predictor	Coefficient	SE	Lower CI	Upper CI	*p* value
CLIMATIC ZONE					0.022
- Temperate	0.299	0.063	0.176	0.422	
- Tropical	0.271	0.072	0.130	0.412	
DIET					0.018
- Carnivore	0.299	0.063	0.176	0.422	
- Herbivore	0.312	0.078	0.160	0.464	
- Omnivore	0.304	0.076	0.156	0.452	
CLUTCH MEAN	-0.054	0.005	-0.063	-0.045	< 0.001

Table 3(on next page)

Phylogenetic Generalized Least Squares scores of variables predicting fecundity in turtles

Diet and egg size predict fecundity in turtle species. SE, standard errors. CI, confidence intervals

Predictor	Coefficient	SE	Lower CI	Upper CI	*p* value
CLIMATIC ZONE					0.531
- Temperate	5.235	0.969	3.337	7.134	
- Tropical	4.888	1.111	2.712	7.065	
DIET					< 0.001
- Carnivore	5.235	0.969	3.337	7.134	
- Herbivore	4.815	1.199	2.466	7.165	
- Omnivore	4.413	1.165	2.131	6.695	
EGG SIZE	-9.525	0.901	-11.291	-7.760	< 0.001