The evolution of reproductive strategies in turtles

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Abstract

The variation in egg and clutch 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 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. We hypothesized that egg and clutch characteristics are influenced by a number of factors, including physiological and ecological constrains, and that reproductive traits in Testudines evolved independently ~~several times in distantly-related clades.~~ We find evidence that turtles explored different reproductive strategies, with several instances of 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, Starck et al., 2021) 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 associated to K/r strategies, has led to several inconclusive discussions (Smith and Fretwell, 1974; Congdon and Gibbons 1987; Wilbur and Morin, 1988; 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 in 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, such as habitat and biogeography (Hofmeyr et al., 2005; Macip-Ríos et al., 2012, 2013), phylogenetic distribution and/or physiology (Bowden et al., 2004, Cordero, 2021).

~~Available~~ evidence supports the idea that large-bodied turtles 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 each extant turtle genus, in order to identify trends in reproductive strategies and investigate potential factors that influence clutch and egg traits. 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 turtle species from different clades converging in their reproductive strategies? 3) Do ecological factors (such as distribution, and diet) influence egg/clutch characteristics? We hypothesize that reproductive traits in turtles evolved independently several times ~~across clades that are not directly related~~, which can be considered evidence of convergent evolution ~~in their reproductive strategies~~. Furthermore, we hypothesize that egg and clutch characteristics follow the predictions of egg size theory. Such characteristics are influenced by several factors, including ecological and phylogenetic constraints, such as diet, geographical distribution and habitat.

Materials and Methods

We collected morphological (carapace size), ecological (climatic zone and diet) and reproductive data (egg size, clutch size, and number of clutches per year) 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 (Supplemental Information, Appendix S2). 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).

Are reproductive traits correlated as predicted by egg size theory?

In order to explore the correlation 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 used the function *evolvcv.lite()* from the R package ~~Phytools~~ (Revell, 2012). This function fits different evolutionary models to a tree to test if two different traits are correlated with the states assigned to a phylogeny (Revell et al., 2021). We pruned the phylogeny proposed by Pereira et al. (2017) to match our dataset (Supplemental Information, Appendix S1). ~~The pruned phylogeny was used in all subsequent analyses.~~

We assigned traits to the phylogeny based on clutch size, using the function *make.simmap()* from phytools R package: A. from 1 to 4 eggs, B. from 5 to 29 eggs, and C. 30 or more eggs. These groupings were arbitrarily chosen to represent discrete traits of maximal fecundity among turtles. As continuously valued traits, we used egg length/carapace as a proxy for relative egg size (ESI) and egg length/egg width as a proxy for egg “shape” (ESH). Model Hierarchy selection was based on best-supported log-likelihood (Log-L), and Akaike information criterion (AIC).

Do turtles have convergent reproductive strategies?

To test the hypothesis of convergence on reproductive traits among turtles, we used the function *search.conv()*, from the R package RRphylo (Castiglione et al., 2019). This function calculates the probability of two or more state-assigned species being convergent based on the angle of their distribution in the phylogeny (see Castiglione et al., 2019 for details).

We assigned states based on clutch size (Supplemental Information, Appendix S1), assuming that turtles with larger clutches tend to produce rounder eggs and possess large body size, while turtles that produce small clutches tend to show elongated eggs and have a small body size. We ran the analysis in three different ways, testing if: A. cryptodirans and pleurodirans with large clutches (over 30 eggs) converge in their reproductive traits, B. cryptodirans and pleurodirans with small clutches (up to four eggs) converge in their reproductive traits, and C. turtles with small clutches (up to four eggs) and large clutches (over 30 eggs) diverge in their reproductive traits. The divergency test is actually one more convergency test, but between the reproductive traits hypothesized to diverge.

Do ecological factors influence egg/clutch characteristics?

In order to estimate which ecological factors influence ~~the distribution of~~ reproductive traits, ~~considering the phylogenetic relationships among Testudines~~, we ran two different phylogenetic generalized least square (PGLS) models ~~(Grafen, 1989; Pagel, 1997, 1999; Rohlf, 2001; Martins and Housworth, 2002~~). 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 egg size 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.

We used Pereira's et al. (2017) pruned tree in both analyses 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 (Mundry, 2014), as they presented high residual values. ~~Models were constructed using information about climatic zone (temperate or tropical) and diet (herbivore, omnivore, or carnivore) of each turtle species (Supplemental Information, Appendix S2~~). Collinearity between ~~pairwise~~ categorical predictors was tested using chi-squared tests (Mundry, 2014; R Core Team, 2020). We used maximum likelihood and Pagel correlation to fit both PGLS, using the function *gls()* of the package nmle (Pinheiro and Bates, 2020).

For model inference, we compared each selected PGLS with its null model (Symonds and Blomber, 2014; Mundry, 2014) and tested for each predictor's significance using the function *anova()* (R Core Team, 2020). 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*; Ives and Li, 2019). We also calculated each predictor's coefficient and its 95% confidence intervals using the PGLS scores table and the function *confint()*, respectively (R Core Team, 2020).

Results

The best fitting among all models used to test correlation among reproductive traits (highest log-likelihood scores, table 1) was the "common rates, common correlation" model. Both traits (ESH and ESI) correlate with the distribution of mapped traits (number of eggs per clutch) in the tree, with the same evolutionary rates.

The *search.conv* analysis revealed significative results for both convergency tests (p = 0.001, figure 2 A and B). The divergency test failed to find any signs of convergency (p = 1.0, figure 2 C). The circular plots revealed small mean angles between states (blue lines, figure 2, bottom row).

In our test of the influence of ecological factor over egg/clutch characteristics, 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*) 71% of the variation in the egg size. Diet, the size of the egg, and climatic zone were all predictors of fecundity in turtles (Table 3; Fig. 4). The selected model explains (*R2 lik*) 62% of the variation in fecundity among turtle species.

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 analysis is consistent with the egg size theory. The selected model reflects a tendency for the selected traits (fecundity, ESI and ESH) to coevolve (Revell et al., 2021). Moreover, 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) through the function *search.conv()* (Castiglione et al., 2019). All three tests followed our hypotheses, demonstrating that animals from different lineages are converging to similar reproductive strategies.

Additionally, diet and climatic zone were the only factors 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 (similar results have been reported for specific turtle clades in previous works, see Macip-Ríos et al. (2017) for an example in Kinosternidae). These results might be influenced by the broad availability of resources in tropical areas, enabling 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 first analysis on egg and clutch correlations. 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 many species show average values, but the high significance of the convergency tests ($P=0.001$) supports our hypothesis.

In addition to the traits tested in the present work, other factors might play important roles in egg and clutch strategies of turtles and could contribute to 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), morphological constraints (Lovich et al., 2012), 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 mentioned by Nussbaum (1987), the safe harbor hypothesis suggests that parental care makes the embryonic stage the safest harbor, favoring egg size to increase in species with parental care, and consequently decreasing the duration of sequential stages with higher risk. 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), terrestrial species lay larger eggs in smaller clutches compared to freshwater or marine species, but this is a statistically confounded association because of the fact that turtle families represent 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, Thompson et al., 2021). 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, that converged to similar reproductive strategies.

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 Arrau 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

~~Convergency~~ and ~~divergency~~ tests for reproductive traits among turtles

The PC plots on the top row were obtained by performing PCAs of the species’ morphological (carapace size) and reproductive data (egg width and egg length). Colored convex hulls represent species belonging to different states. The first two plots represent ~~convergency~~ tests between pleurodirans (state A) and cryptodirans (state B) that produce clutches containing up to four eggs (plot A) and over 30 eggs (plot B). Plot C represent a divergency test among species that produce small and large clutches, containing over 30 eggs (state A) and up to four eggs (state B). The circular plots on the bottom represent the mean θreal angle between tested states (blue lines) and the range of θrandom angles (gray shaded area) of the same states. The p-value of the convergence tests are available in each plot.

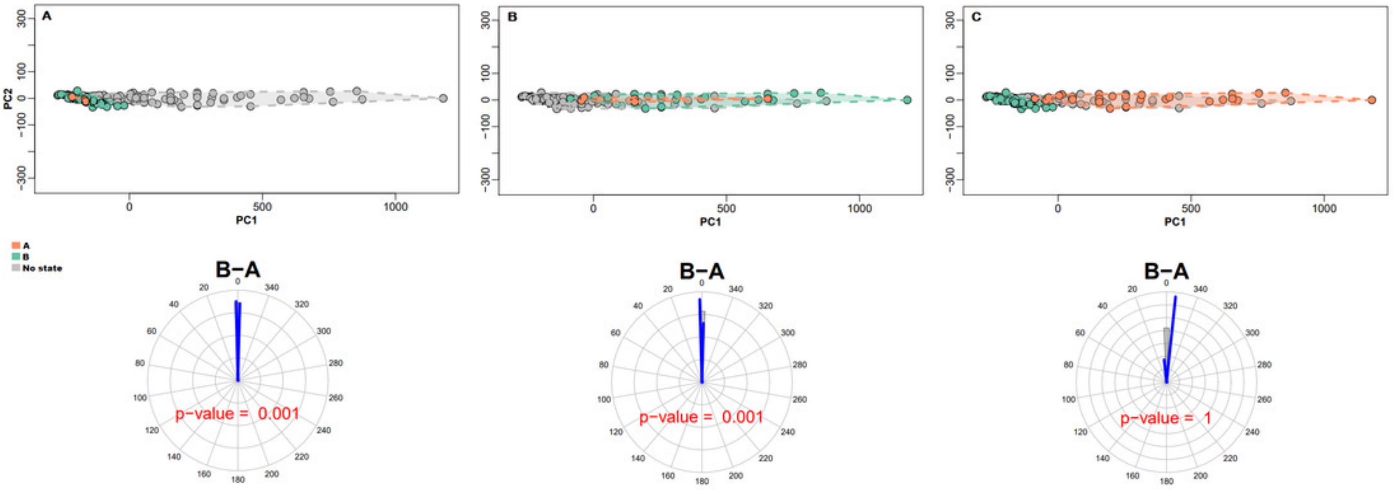

Figure 3

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 (temperate or tropical) and have different diet types (carnivore, herbivore, or omnivore).

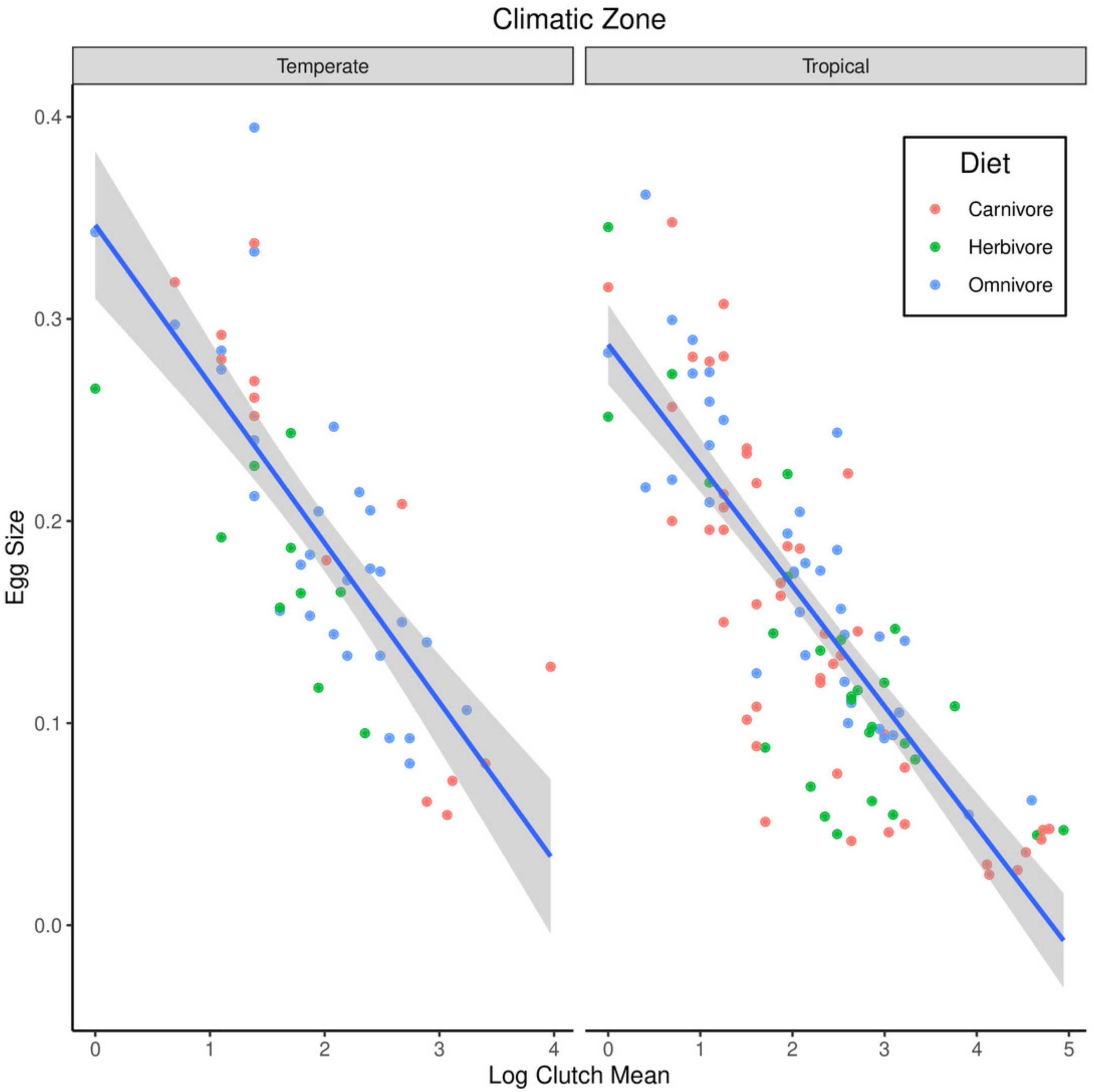
Climatic Zone
Temperate
Tropical
Diet
Carnivore
Herbivore
Omnivore
Egg Size
0.0
0.1
0.2
0.3
0.4
Log Clutch Mean
0
1
2
3
4
5

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) or turtle species that occupy different climatic zones (temperate or tropical) and have different diet types (carnivore, herbivore, or omnivore).

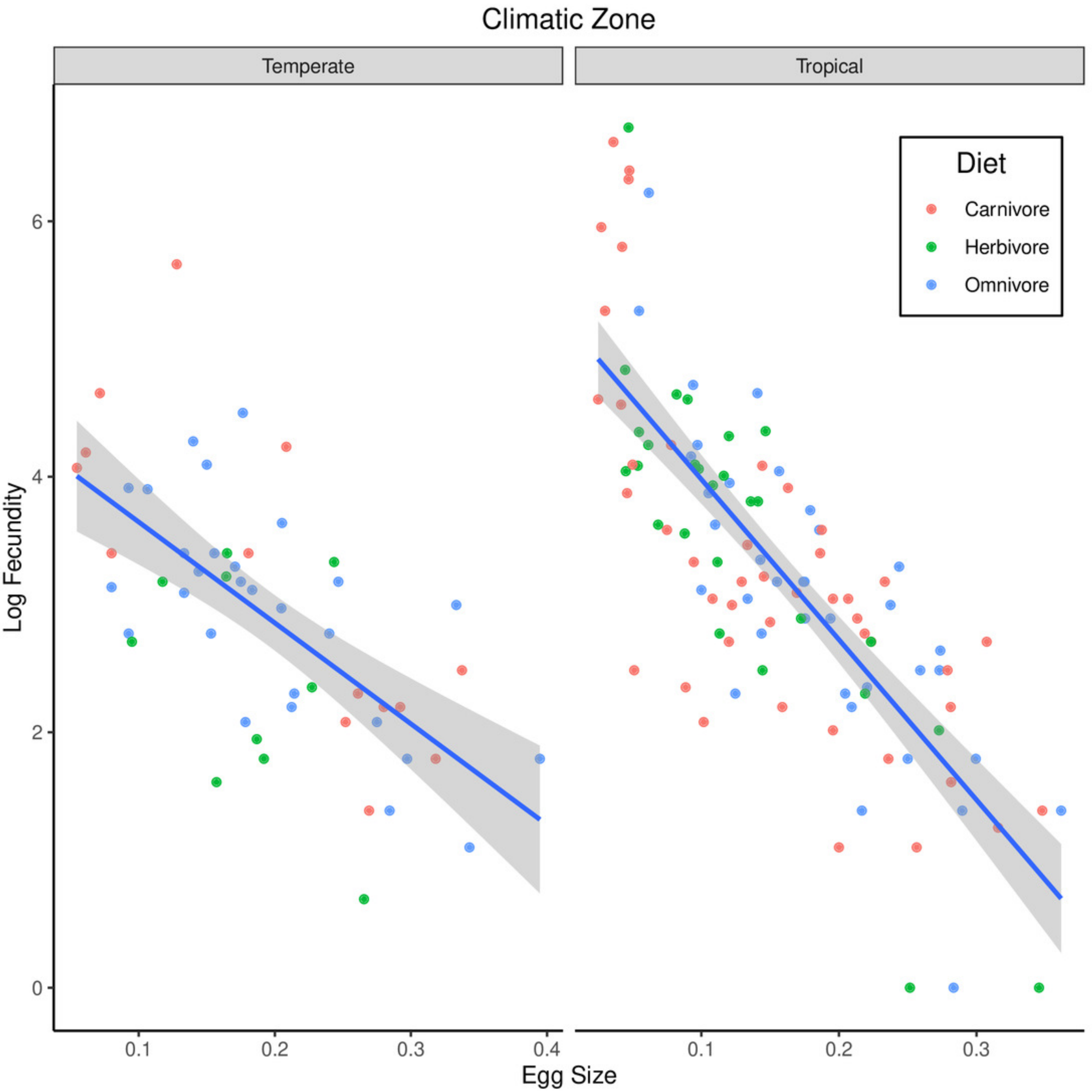
Climatic Zone
Temperate
Tropical
Diet
● Carnivore
● Herbivore
● Omnivore
Log Fecundity
0
2
4
6
0.1
0.2
0.3
0.4
0.1
0.2
0.3
Egg Size

Table 1(on next page)

Hierarchical models of evolutionary correlation among reproductive traits in turtles

Model description, model parameter estimates (K), log-likelihood (Log-L), and Akaike information criterion (AIC) for four multivariate Brownian evolution model fits to egg and clutch data. The best-supported model is highlighted in bold.

Model	K	Log-L	AIC
common rates, common correlation	**5**	**163.8606**	**-317.7212**
different rates, common correlation	9	-585.9692	1189.9384
common rates, different correlation	7	22.3826	-30.7652
No common structure	11	107.204	-192.408

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					< 0.001
- Temperate	0.307	0.018	0.272	0.342	
- Tropical	0.295	0.026	0.244	0.346	
DIET					< 0.001
- Carnivore	0.307	0.018	0.272	0.342	
- Herbivore	0.292	0.031	0.232	0.353	
- Omnivore	0.318	0.030	0.260	0.376	
CLUTCH MEAN	-0.060	0.004	-0.068	-0.051	< 0.001

Table 3(on next page)

Phylogenetic Generalized Least Squares scores of variables predicting fecundity in turtles

Climatic zone, 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					0.021
- Temperate	4.917	0.361	4.210	5.625	
- Tropical	4.986	0.501	4.003	5.968	
DIET					0.006
- Carnivore	4.917	0.361	4.210	5.625	
- Herbivore	5.006	0.582	3.865	6.148	
- Omnivore	5.108	0.564	4.003	6.212	
EGG SIZE	-10.577	0.900	-12.341	-8.812	< 0.001