The evolution of reproductive strategies in turtles

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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.

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Abstract

Optimal egg 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. Turtles offer a rich subject of investigation, given the ecological diversity of the group. Evidence supports the idea that large-bodied turtles tend to produce larger clutches with small and round eggs, while smaller species produce small clutches with large and elongated eggs. Trends in egg and clutch characteristics also seem to be influenced by ecological factors. Until now, a comprehensive analysis exploring reproductive characteristics across all genera of living turtles was missing. Our goals were to investigate whether egg and clutch size follow the predictions of egg size theory, if there are convergent reproductive strategies, and identify ecological factors that influence clutch and egg traits. Here, we report results of analyses on reproductive characteristics across all clades of living turtles, including at least one representative of each extant turtle genus. Using phylogenetic methods, we tested the covariance among reproductive traits and if they are convergent among different turtle lineages. We also estimated which ecological factors influence these traits. Our analyses are consistent with the optimal egg size theory, with evidences for convergent evolution. We also verified that diet and geographical distribution influence the size of eggs and clutches. We conclude that egg and clutch traits in Testudines evolved independently several times across non-directly related clades that converged to similar reproductive strategies.

Introduction

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 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 discussions without a consensus about the distribution or reasons of such correlations (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 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 resource availability and temperature influenced by habitat and biogeography (Hofmeyr et al., 2005; Macip-Ríos et al., 2012, 2013), phylogenetic distribution and/or physiology (Bowden et al., 2004, Cordero, 2021).

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 – therefore being more suitable for warmer areas. 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 egg width. Moll (1979) argued that spherical eggs occupy space more efficiently than elongated eggs, thereby allowing the fit of larger clutches in the abdominal cavity.

Many trends in egg and clutch characteristics also seem to be influenced by ecological factors. Rachmansah et al., (2020) suggested that the broad access to resources in tropical areas, supports larger-bodied taxa to produce more eggs. Craven et al., (2008) proposed that resource availability and type of diet might play a role in egg nutrition (Craven et al., 2008). Spencer and Janzen (2011), advocate that higher mean temperature of tropical areas may influence embryo metabolism and favor earlier hatching – favoring the production of more clutches per year.

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, ~~which can be considered evidence of convergent evolution~~. Furthermore, we hypothesize that egg and clutch characteristics follow the predictions of egg size theory. Such characteristics are influenced by several ecological factors (e.g., carnivores tend to produce bigger eggs and tropical species tend to produce bigger clutches).

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. We used Google scholar ~~database~~ to perform an electronic search using different combinations of the key words “Egg size”, “turtle reproduction”, “breeding”, “nest”, “clutch size”, “egg width”. Studies from all dates were considered, as evolutionary characteristics of species do not usually change within the relevant time for a literature search. Only full-text reports in English, Spanish and Portuguese were considered. Study eligibility was assessed by one investigator. A secondary search was conducted on the reference list of these publications as well as on the list of publications that have cited the previous accessed one. The search continued until the limit of four articles containing information on the same ecological data for each species. The search was conducted following PRISMA (Moher et al., 2011) guidelines (Supplemental Information, Appendix S2).

Data used was based on a combination of the information available (e.g., smallest and biggest clutch sizes reported, even if from different sources) or based on the most common attribution for each species (*e.g.,* species that are found both in land and water but most commonly in water were addressed with this kind of habitat). Data from captivity was considered as the

characteristics of interest are mostly supposed to be inheritable and we considered possible bias to be irrelevant.

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). We pruned the phylogeny proposed by Pereira et al. (2017) to match our dataset (Supplemental Information, Appendix S1) and used it in all the following analyses. Although this is not the most recent phylogeny available, it was the one with the biggest overlap with our dataset.

Are reproductive traits correlated as predicted by egg size theory?

In order to explore the correlation ~~of~~ reproductive parameters (egg width, egg length, and clutch size) 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~~ covariance matrices to a tree to test if ~~there is an evolutionary tendency for~~ two traits to co-evolve, affected by two or more regimes ~~assigned to the tree.~~ (Revell et al., 2021).

We mapped the phylogeny through ancestral state reconstruction using ~~traits based on clutch size~~ (Fig. 2A), and 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 ~~based on what we understand~~ to best reflect the biological meaning of the data, and represent discrete traits of maximal clutch size 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). The choice for the model that better explains our analysis was based on likelihood ratio test (Log-L), and Akaike information criterion (AIC; Akaike, 1974). We also plotted the data in a phylomorphospace, to support visualization (Fig. 2B).

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).

Species at the tree terminals are represented by a vector based on multivariate phenotypic data. This function calculates the angle between them (computed as the inverse cosine of the ratio product, and the product of vectors sizes), which represents the correlation coefficient between the two vectors to represent a measure of phenotypic resemblance. Small angles between vectors imply similar phenotypes, while angles around 90˚ and 180˚ represent dissimilar and opposing phenotypes, respectively (Castiglione et al., 2019). To verify convergence, we test if the differences between groups are smaller than expected considering their phylogenetic distance. The function can be used to test convergence either ~~in~~ entire clades or among species assigned ~~under~~ different ~~estates~~. Considering that our hypothesis of convergence includes species scattered along the phylogeny in a complex evolutionary history, a search without predetermined groups would have to compare the angles of phenotypic divergency between all species combinations (oppositely to comparisons among clades with higher taxonomic level such as families). Therefore, we ~~decided for~~ assigning states based on clutch size (Supplemental Information, Appendix S1) to ensure computational viability. This decision also follows the methodology implemented in the previous question ~~(if traits correlate following the egg size theory~~), and is based on the observations that turtles with larger clutches tend to produce smaller and rounder eggs, and possess large body size, while turtles that produce small clutches tend to show larger, elongated eggs, and have a small body size.

We first ran the analysis by testing if cryptodirans and pleurodirans converge in their reproductive strategies. To do that, we assigned each species to one of six ~~different~~ states: pleurodirans that produce clutches containing A. bellow 5 eggs, B. from 5 to 29 eggs, and C. 30 or more eggs; or cryptodirans that produce clutches containing C. bellow 5 eggs, D. from 5 to 29 eggs, and E. 30 or more eggs. We divided the same characters into two different states based on suborder (A and D, B and E, and C and F) in order to follow the analysis requirements. As it tests the convergence of groups distantly related, character states must be considered different. We used the suborders Cryptodira and Pleurodira to assign different characters as they are the most comprehensive taxonomic levels among turtles. By doing this, we tested if species in between these suborders are converging among three different states based on clutch size (small, medium and large). The convergence test between different traits in different clades represent the null models (they are not expected to converge).

Later, we ran a second analysis, without any separation among turtles, to test if species with small clutches (up to four eggs) and large clutches (over 30 eggs) diverge in their reproductive traits. The divergency test is nothing more than another convergence test, but opposing the reproductive traits hypothesized to diverge, which also works as our null model (lack of convergence). Both tests were simulated 1000 times and tips under the focal states were randomly removed until clustering remained with only three tips.

Do ecological factors influence egg/clutch characteristics?

In order to estimate which ecological factors influence reproductive traits, we ran two different phylogenetic generalized least square (PGLS) models (Grafen, 1989; ~~Rohlf, 2001;~~ Martins and Housworth, 2002). In the first analysis, we tested how climatic zone, diet and the log clutch mean predict egg size. 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 per 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) predicted the fecundity in turtles. We log-transformed the fecundity variable in order to achieve homoscedasticity and normality of residuals (Mundry, 2014).

Multicollinearity between categorical predictors was tested using chi-squared tests (Mundry, 2014; R Core Team, 2020). We used maximum likelihood and Pagel's lambda model (Pagel, 1997; 1999) to control for phylogenetic signal when fitting both PGLS. We used the function *gls()* of the package nmle (Pinheiro and Bates, 2020).

For model inference, we compared each selected PGLS with its null model and tested for each predictor's significance (Symonds and Blomber, 2014; Mundry, 2014). We 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 "different rates, common correlation" model. Egg traits (ESH

and ESI) coevolve and correlate in the same way with the regimes of traits mapped in the tree (number of eggs per clutch; R=0.567), although with different evolutionary rates. Different regimes of clutch size occupy different regions of the morphospace (Fig. 2B).

The first convergence analysis revealed significative results in tests performed against same characters between different pleurodirans and cryptodirans ($p = 0.001$, Table 2, ~~in green~~). Additionally, the analysis also indicated significative results for convergence tests between medium sized clutches (B and E) and other size clutches (A, C, D, and F), although only between different suborders ($p = 0.001$, Table 2, ~~in bold~~). The divergency test failed to find any signs of convergence ($p = 1.0$) between turtles with small and large clutches.

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 3; Fig. 3). Egg size and climatic zone were significant predictors of fecundity in turtles (Table 4; Fig. 4).

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 evolutionary history. 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).

The results of our analysis are consistent with the predictions of the egg size theory. The selected model reflects a tendency for the traits “egg size” and “egg shape” to positively coevolve, while both are inversely correlated to “clutch size”. Moreover, based on the distribution of characters along the tree and in the phylomorphospace, ~~we can assert that~~ these patterns evolved independently and recurrently along the diversification of turtles. 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). Turtles of the~~ Pleurodira and Cryptodira converge in all three different reproductive strategies tested.

Because all traits used in the convergence tests are continuous, without any clear break in the patterns, we also recovered significative results among medium size clutches and small or big size clutches between different "suborders". The fact that the same results were not recovered within suborders, is an indication that this result is a type I error. Because "suborder" is a highly inclusive taxonomic rank, the phenotypic differences between groups are considered smaller than expected considering their evolutionary distance. When the same traits are tested within the same "suborder", their differences are not less than expected and, therefore, are not pointed as convergent.

The divergence test between small and large clutches among all turtles indicated these traits are indeed divergent, independently of the taxonomic rank. Therefore, evidence indicates that turtles are converging to at least two – most probably three – different egg and clutch strategies, with continuous traits that prevent a clear differentiation among them. Nevertheless, these traits follow the egg/clutch size theory.

Although our analyses provided evidence for convergence among different turtle clades, they do not explain the reasons for such convergence, ~~namely the pressures selecting similar behaviors in groups with distant evolutionary origins~~. With the PGLS analyses, we were able to specify some of the ecological pressures affecting egg and clutch characteristics. Climatic zone is the only factor to partially explain the correlations in both analyses. Egg size is also influenced by diet with herbivores producing relatively smaller eggs. 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). Additionally, high protein intake seems to stimulate egg production in turtles and other animals (Watanabe et al., 1984; Bjorndal, 1985). 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).

Since many turtle families are spread across different geographic areas, in different climatic zones and with specific available resources (e.g., Emydidae across the Americas), close related species are subjected to extremely different ecological pressures. Sympatric distant related species, however, suffer similar ecological pressures and, therefore, tend to occupy similar ecological niches despite their intrinsic phylogenetic distance.

~~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 clutch size, demonstrating that reproductive traits are correlated as predicted by egg size theory.

Based on these results, we conclude 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 convergence tests (*P=0.001*) supports our hypothesis~~.

In addition to the traits tested in the present study, other factors may 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 ~~shouldn't~~ be forgotten when interpreting this complex scenario (see Kluge, 2005 and Losos, 2011 for a review of the role of convergent evolution in inferring adaptations). For instance, little is known about many of the aspects that influence the reproductive characteristics within Testudines. These 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 Testudinidae, 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 distribution of these strategies is associated with groups that colonized 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,

296 which makes hypotheses based on niche adaptations greatly speculative (Popper, 1982; Stayton,
297 2015b).

298 Conclusions

299 We conclude that egg and clutch traits in Testudines evolved independently several times across
300 non-directly related clades that converged to similar reproductive strategies.

301 Egg and clutch characteristics follow the trade-offs predicted by egg size theory and are
302 influenced by ecological factors. Climatic zone plays an important role in the distribution of
303 reproductive characteristics among turtles, and diet influences egg size.

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308 References

309 Agha, M., Lovich, J.E., Ennen, J.R. and Wilcox, E., 2013. Nest-guarding by female Agassiz's
310 desert tortoise (*Gopherus agassizii*) at a wind-energy facility near Palm Springs, California. The
311 Southwestern Naturalist, 58(2), pp.254-257.

312 Akaike, H., 1974. A new look at the statistical model identification. IEEE transactions on
313 automatic control, 19(6), pp.716-723.

314 Bartoń, K., 2015. Multi-model inference, version 1.15.6. Available at https:// cran.r-
315 project.org/web/packages/MuMIn/index.html. R Foundation for Statistical Computing, Austria.

316 Battistella, T., Cerezer, F., Bubadué, J., Melo, G., Graipel, M. and Cáceres, N., 2019. Litter size
317 variation in didelphid marsupials: evidence of phylogenetic constraints and adaptation.
318 Biological Journal of the Linnean Society, 126(1), pp.40-54.

319 Bjorndal, K.A., 1985. Nutritional ecology of sea turtles. Copeia, pp.736-751.

320 Bowden, R.M., Harms, H.K., Paitz, R.T. and Janzen, F.J., 2004. Does optimal egg size vary with
321 demographic stage because of a physiological constraint? Functional Ecology, pp.522-529.

322 Brockelman, W.Y., 1975. Competition, the fitness of offspring, and optimal clutch size. The
323 American Naturalist, 109(970), pp.677-699.

324 Burnham, K.P., and Anderson, D.R., 2002. Model Selection and Multimodel Inference – A
325 Practical Information-Theoretic Approach. Springer, USA.

326 Burnham, K.P., Anderson, D.R. and Huyvaert, K.P., 2011. AIC model selection and multimodel
327 inference in behavioral ecology: some background, observations, and comparisons. Behavioral
328 ecology and sociobiology, 65(1), pp.23-35.

329 Castiglione, S., Serio, C., Tamagnini, D., Melchionna, M., Mondanaro, A., Di Febbraro, M., ... &
330 Raia, P., 2019. A new, fast method to search for morphological convergence with shape data.
331 PloS one, 14(12), e0226949.

332 Cordero, G. A., 2021. Disentangling the correlated evolution of body size, life history, and
333 ontogeny in miniaturized chelydroid turtles. Evolution & Development, e12386.

334 Clark, P.J., Ewert, M.A. and Nelson, C.E., 2001. Physical apertures as constraints on egg size
335 and shape in the common musk turtle, *Sternotherus odoratus*. Functional Ecology, pp.70-77.

336 Congdon, J.D. and Gibbons, J.W., 1987. Morphological constraint on egg size: a challenge to
337 optimal egg size theory? Proceedings of the National Academy of Sciences, 84(12), pp.4145-
338 4147.

339 Craven, K.S., Parsons, J., Taylor, S.A., Belcher, C.N. and Owens, D.W., 2008. The influence of
340 diet on fatty acids in the egg yolk of green sea turtles, Chelonia mydas. Journal of Comparative
341 Physiology B, 178(4), pp.495-500.

342 Deeming, D.C. and Birchard, G.F., 2007. Allometry of egg and hatchling mass in birds and
343 reptiles: roles of developmental maturity, eggshell structure and phylogeny. Journal of Zoology,
344 271(1), pp.78-87.

345 Deeming, D.C. and Ruta, M., 2014. Egg shape changes at the theropod–bird transition, and a
346 morphometric study of amniote eggs. Royal Society open science, 1(3), p.140311.

347 Elgar, M. and Heaphy, L.J., 1989. Covariation between clutch size, egg weight and egg shape:
348 comparative evidence for chelonians. Journal of Zoology, 219(1), pp.137-152.

349 Ferrara, C.R., Vogt, R.C. and Sousa-Lima, R.S., 2013. Turtle vocalizations as the first evidence
350 of posthatching parental care in chelonians. Journal of Comparative Psychology, 127(1), p.24.

351 Gibbons, J.W., 1982. Reproductive patterns in freshwater turtles. Herpetologica, pp.222-227.

352 Godfray, H.C.J., Partridge, L. and Harvey, P.H., 1991. Clutch size. Annual Review of Ecology
353 and Systematics, 22(1), pp.409-429.

354 Grafen, A., 1989. The phylogenetic regression. Phil Trans R Soc B, 326, pp.119–157.

355 Gray, J.E., 1863. Observations on the box tortoises, with the descriptions of three new Asiatic
356 species. In Proceedings of the zoological Society of London (Vol. 1863, pp. 173-179).

357 Hedrick, A.R., Klondaris, H.M., Corichi, L.C., Dreslik, M.J. and Iverson, J.B., 2018. The effects
358 of climate on annual variation in reproductive output in Snapping Turtles (*Chelydra serpentina*).
359 Canadian Journal of zoology, 96(3), pp.221-228.

360 Hofmeyr, M.D., Henen, B.T. and Loehr, V.J., 2005. Overcoming environmental and
361 morphological constraints: egg size and pelvic kinesis in the smallest tortoise, *Homopus*
362 *signatus*. Canadian Journal of Zoology, 83(10), pp.1343-1352.

363 Hughes, E.J. and Brooks, R.J., 2006. The good mother: does nest-site selection constitute
364 parental investment in turtles?. Canadian Journal of Zoology, 84(11), pp.1545-1554.

365 Iverson, J.B., 1992. Correlates of reproductive output in turtles (Order Testudines).
366 Herpetological Monographs, pp.25-42.

367 Iverson, J.B., Balgooyen, C.P., Byrd, K.K. and Lyddan, K.K., 1993. Latitudinal variation in egg
368 and clutch size in turtles. Canadian Journal of Zoology, 71(12), pp.2448-2461.

Iverson, J.B., Lindeman, P.V. and Lovich, J.E., 2019. Understanding reproductive allometry in turtles: A slippery “slope”. Ecology and evolution, 9(20), pp.11891-11903.

Janzen, F.J. and Warner, D.A., 2009. Parent–offspring conflict and selection on egg size in turtles. Journal of evolutionary biology, 22(11), pp.2222-2230.

Jetz, W., Sekercioglu, C.H. and Böhning-Gaese, K., 2008. The worldwide variation in avian clutch size across species and space. PLoS Biol, 6(12), p.e303.

Johnson, J. B. and Omland, K.S., 2004. Model selection in ecology and evolution. Trends in Ecology and Evolution 19, pp.101–108.

Kaplan, R.H. and Salthe, S.N., 1979. The allometry of reproduction: an empirical view in salamanders. The American Naturalist, 113(5), pp.671-689.

Kluge, A.G., 2005. Testing lineage and comparative methods for inferring adaptation. Zoologica Scripta, 34, 653-663.

Kuchling, G., 1999. The reproductive biology of the Chelonia. Springer Science & Business Media.

Le, M., McCord, W.P. and Iverson, J.B., 2007. On the paraphyly of the genus *Kachuga* (Testudines: Geoemydidae). Molecular phylogenetics and evolution, 45(1), pp.398-404.

Li, Z.H.A.O., Cheng, C.H.E.N. and Liao, W.B., 2017. No evidence for trade-off between clutch size and egg size in the spot-legged treefrog (*Polypedates megacephalus*). North-Western Journal of Zoology, 13(1).

Losos, J.B., 2011. Convergence, adaptation, and constraint. Evolution, 65, 1827-1840.

Lovich, J. E., Madrak, S. V., Drost, C. A., Monatesti, A. J., Casper, D., and Znari, M., 2012. Optimal egg size in a suboptimal environment: reproductive ecology of female Sonora mud turtles (*Kinosternon sonoriense*) in central Arizona, USA. Amphibia-Reptilia, 33(2), 161-170.

Macip-Ríos, R., Brauer-Robleda, P., Casas-Andreu, G., de Lourdes Arias-Cisneros, M., & Sustaita-Rodríguez, V. H., 2012. Evidence for the morphological constraint hypothesis and

394 optimal offspring size theory in the Mexican mud turtle (*Kinosternon integrum*). Zoological
395 Science, 29(1), pp.60-65.

396 Macip-Ríos, R., Sustaita-Rodriguez, V.H. and Casas-Andreu, G., 2013. Evidence of pelvic and
397 nonpelvic constraint on egg size in two species of *Kinosternon* from Mexico. Chelonian
398 Conservation and Biology, 12(2), pp.218-226.

399 Macip-Ríos, R., Ontiveros, R. N., Sánchez-León, A. T., and Casas-Andreu, G., 2017. Evolution
400 of reproductive effort in mud turtles (Kinosternidae): the role of environmental predictability.
401 Evolutionary Ecology Research, 18(5), 539-554.

402 Martins, E. P. and Housworth, E. A., 2002. Phylogeny shape and the phylogenetic comparative
403 method. Syst Biol, 51, pp.873–880.

404 Moher, D., Altman, D.G., Liberati, A. and Tetzlaff, J., 2011. PRISMA statement. Epidemiology,
405 22(1), p.128.

406 Moll, E.O., 1979. Reproductive cycles and adaptations. Turtles: perspectives and research,
407 pp.305-331.

408 Mundry, R. 2014. Statistical Issues and Assumptions of Phylogenetic Generalized Least Squares.
409 In: Garamszegi, L.Z. Modern Phylogenetic Comparative Methods and Their Application in
410 Evolutionary Biology. Springer, pp 131-153. doi: 10.1007/978-3-662-43550-2_6.

411 Murray, C.M., Crother, B.I. and Doody, J.S., 2020. The evolution of crocodilian nesting ecology
412 and behavior. Ecology and Evolution, 10(1), pp.131-149.

413 Nussbaum, R.A., 1987. Parental care and egg size in salamanders: an examination of the safe
414 harbor hypothesis. Population Ecology, 29(1), pp.27-44.

415 Pagel, M., 1997. Inferring evolutionary processes from phylogenies. Zool Scripta, 26, pp.331–
416 348.

417 Pagel, M., 1999. Inferring the historical patterns of biological evolution. Nature, 401, pp.877–
418 884.

Pereira, A.G., Sterli, J., Moreira, F.R. and Schrago, C.G., 2017. Multilocus phylogeny and statistical biogeography clarify the evolutionary history of major lineages of turtles. Molecular Phylogenetics and Evolution, 113, pp.59-66.

Popper, K.R., 1982. Science: Conjecture and refutations. Philosophy of science and the occult, pp.104-111.

Pritchard, P.C.H., 1979. Encyclopedia of turtles (p. 876). New Jersey, Estados Unidos: TFH.

R Development Core Team. 2016. R: A Language and Environment for Statistical Computing, Version 3.6.3. Available at http://www.R-project.org/. R Foundation for Statistical Computing, Austria.

R Core Team. 2020. Stats. R package, version 4.0.3.

Rachmansah, A., Norris, D. and Gibbs, J.P., 2020. Population dynamics and biological feasibility of sustainable harvesting as a conservation strategy for tropical and temperate freshwater turtles. PloS one, 15(2), p.e0229689.

Revell, L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods in ecology and evolution, 3(2), pp.217-223.

Revell, L. J., Toyama, K. S., & Mahler, D. L., 2021. A simple hierarchical model for heterogeneity in the evolutionary correlation on a phylogenetic tree. bioRxiv.

Rohlf, F. J., 2001. Comparative methods for the analysis of continuous variables: geometric interpretations. Evolution 55, pp.2143–2160.

Rowe, J.W., 1994. Egg size and shape variation within and among Nebraskan painted turtle (*Chrysemys picta bellii*) populations: relationships to clutch and maternal body size. Copeia, pp.1034-1040.

Santos, R.G., Pinheiro, H.T., Martins, A.S., Riul, P., Bruno, S.C., Janzen, F.J. and Ioannou, C.C., 2016. The anti-predator role of within-nest emergence synchrony in sea turtle hatchlings. Proceedings of the Royal Society B: Biological Sciences, 283(1834), pp.20160697.

444 Symonds, M.R.E.; and Blomberg, S.P. 2014. A Primer on Phylogenetic Generalised Least
445 Squares. In: Garamszegi, L.Z. Modern Phylogenetic Comparative Methods and Their
446 Application in Evolutionary Biology. Springer, pp 105-130. doi: 10.1007/978-3-662-43550-2_5.

447 Smith, C. C., & Fretwell, S. D., 1974. The optimal balance between size and number of
448 offspring. The American Naturalist, 108(962), pp.499-506.

449 Spencer, R.J. and Janzen, F.J., 2011. Hatching behavior in turtles. Integrative and Comparative
450 Biology, 51(1), pp.100-110.

451 Starck, J. M., Stewart, J. R., & Blackburn, D. G., 2021. Phylogeny and evolutionary history of
452 the amniote egg. Journal of Morphology.

453 Stayton, C.T., 2015a. What does convergent evolution mean? The interpretation of convergence
454 and its implications in the search for limits to evolution. Interface focus, 5(6), p.20150039.

455 Stayton, C.T., 2015b. The definition, recognition, and interpretation of convergent evolution, and
456 two new measures for quantifying and assessing the significance of convergence. Evolution,
457 69(8), pp.2140-2153.

458 Symonds, M.R.E., and A. Moussalli. 2011. A brief guide to model selection, multimodel
459 inference and model averaging in behavioural ecology using Akaike's information criterion.
460 Behavioral Ecology and Sociobiology 65:13–21.

461 Thomson, R. C., Spinks, P. Q., and Shaffer, H. B., 2021. A global phylogeny of turtles reveals a
462 burst of climate-associated diversification on continental margins. Proceedings of the National
463 Academy of Sciences, 118(7).

464 Warner, D.A., Jorgensen, C.F. and Janzen, F.J., 2010. Maternal and abiotic effects on egg
465 mortality and hatchling size of turtles: temporal variation in selection over seven years.
466 Functional Ecology, 24(4), pp.857-866.

467 Watanabe, T., Takeuchi, T., Saito, M. and Nishimura, K., 1984. Effect of low protein-high calory
468 or essential fatty acid deficiency diet on reproduction of rainbow trout [*Salmo gairdnerii*].
469 Bulletin of the Japanese Society of Scientific Fisheries (Japan).

Wilbur, H. M., & Morin, P. J., 1988. Life history evolution in turtles. Biology of the Reptilia, 16, pp.387-439.

Wilkinson, L.R. and Gibbons, J.W., 2005. Patterns of reproductive allocation: clutch and egg size variation in three freshwater turtles. Copeia, 2005(4), pp.868-879.

Yu, T.L. and Deng, Y.H., 2020. Geographic variation in maternal investment and trade-offs between egg size and clutch size in an endemic toad of the Qinghai-Tibet plateau. Scientific Reports, 10(1), pp.1-8.

Figure 1

Egg and clutch strategies.

Examples of different strategies: 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

Distribution of egg and clutch traits in the turtle phylogeny.

Different clutch sizes were assigned to three different regimes (small, medium, and large) and mapped to the tree (A); Turtle phylogeny was plotted in a morphospace based on egg size and shape (B).

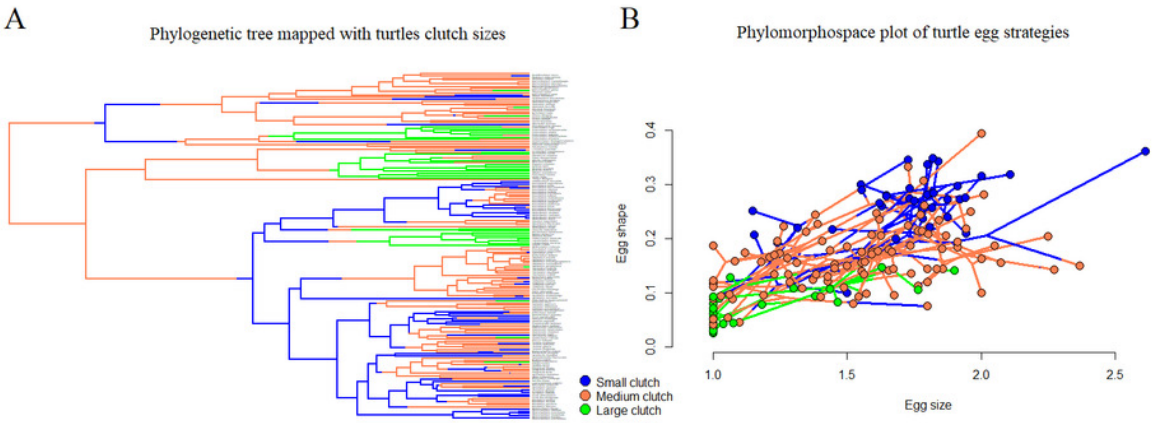

Figure 3

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).

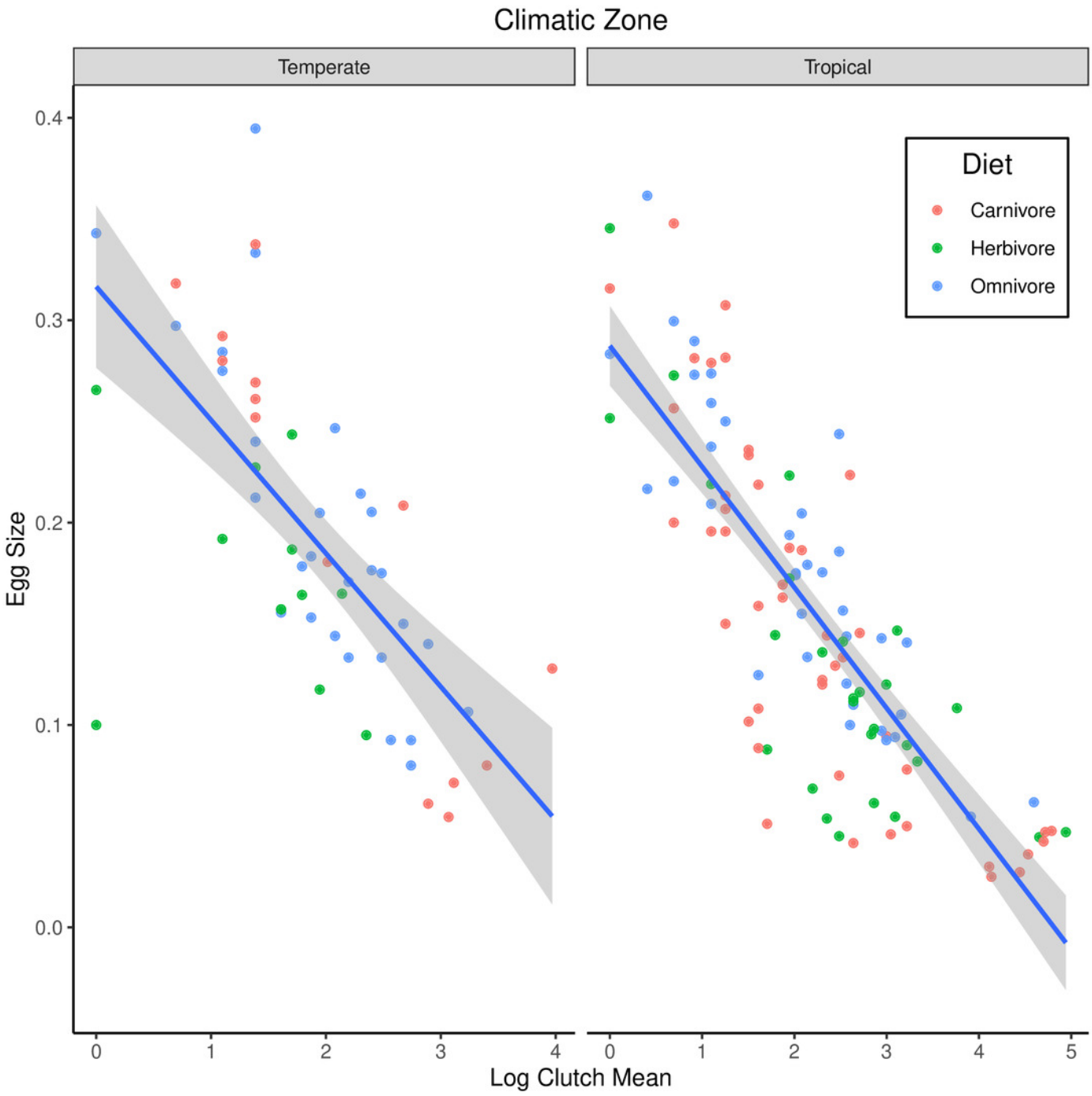
Climatic Zone
Temperate
Tropical
Diet
Carnivore
Herbivore
Omnivore
Egg Size
0.0
0.1
0.2
0.3
0.4
0
1
2
3
4
5
Log Clutch Mean

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 occupy different climatic zones (temperate or tropical).

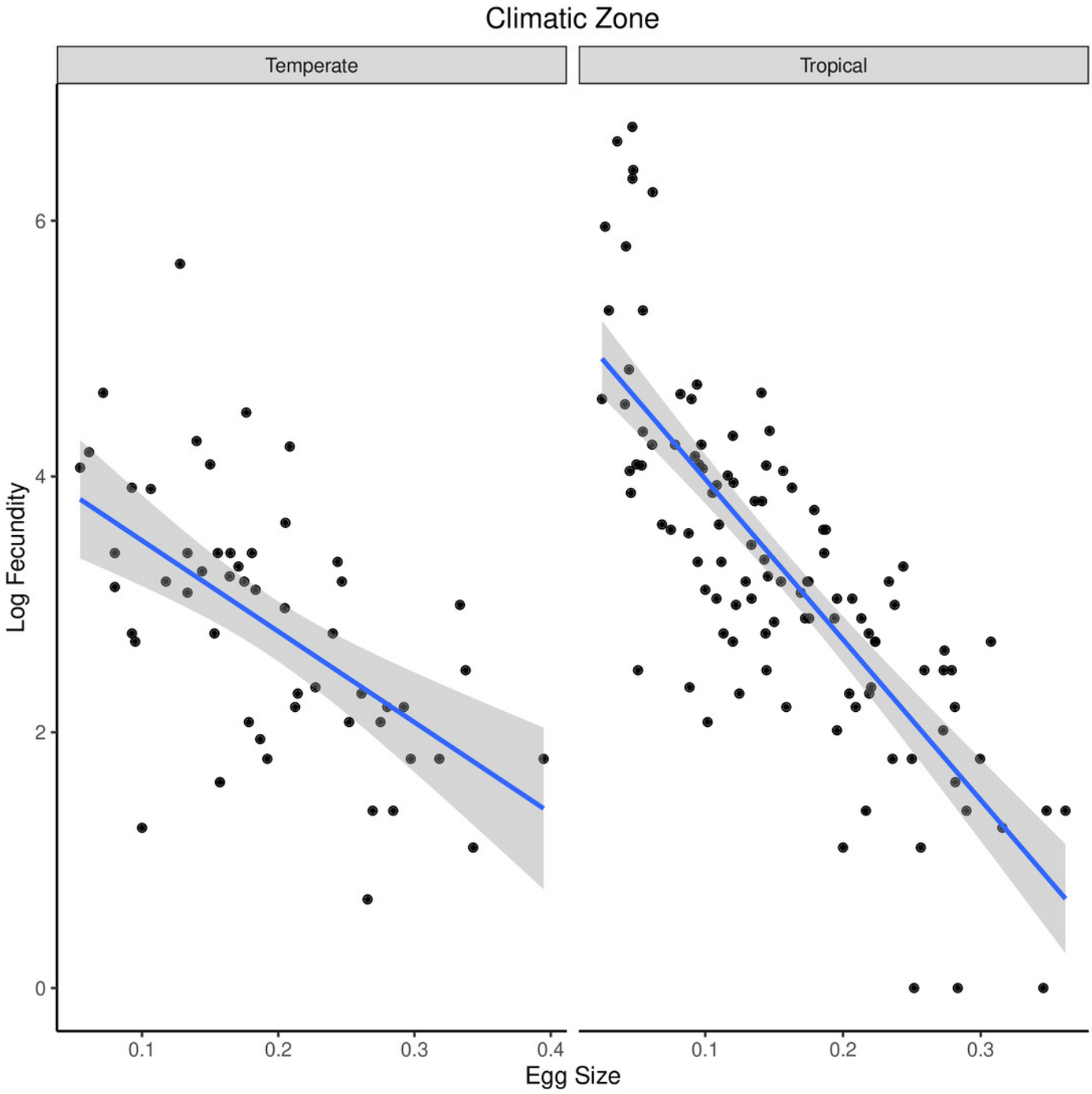
Climatic Zone
Temperate
Tropical
Log Fecundity
Egg Size
0
2
4
6
0.1
0.2
0.3
0.4

Table 1(on next page)

Hierarchical models of evolutionary correlation among reproductive traits in turtles.

Model description, rates of correlation between egg size and three different clutch size groups ($\sigma^2_{1,x}$), rates of correlation between egg shpe and three different clutch size groups ($\sigma^2_{2,x}$), correlation between egg size and egg shape, affected by different regimes of clutch size (R), 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	σ ²1,1	σ ² 1,2	σ ² 1,3	σ ² 2,1	σ ² 2,2	σ ² 2,3	R1	R2	R3	Log(L)	AIC
common rates, common correlation	0.002	-	-	0.0001	-	-	0.519	-	-	199.12	-388.
different rates, common correlation	**0.0043**	**0.0015**	**0.0007**	**0.0002**	**0.0001**	**0**	**0.567**	**-**	**-**	**222.99**	**-427.**
common rates, different correlation	0.0021	-	-	0.0001	-	-	0.356	0.599	0.958	209.02	-404.
No common structure	0.004	0.0015	0.001	0.0002	0.0001	0	0.458	0.586	0.829	224.11	-426.

1

2

Table 2(on next page)

Tests of convergence among different reproductive strategies in turtles.

Letters represent traits based in different clutch sizes: small (bellow 5 eggs), medium (from 5 to 29 eggs), and large (30 or more eggs), for pleurodirans (A, B, and C, respectively), and cryptodirans (D, E, and F, respectively). Tests that presented significative results for convergence (*P=0.001*) are in bold. Tests between same traits and between same suborders are in green.

State 1	State 2	P value
D	E	1
D	F	1
D	A	**0.001**
D	B	**0.001**
D	C	0.482
E	F	0.703
E	A	**0.001**
E	B	**0.001**
E	C	**0.001**
F	A	0.229
F	B	**0.001**
F	C	**0.001**
A	B	0.195
A	C	0.754
B	C	0.133

1

Table 3(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					0.002
- Temperate	0.296	0.019	0.259	0.333	
- Tropical	0.288	0.028	0.234	0.342	
DIET					< 0.001
- Carnivore	0.296	0.019	0.259	0.333	
- Herbivore	0.278	0.031	0.217	0.340	
- Omnivore	0.306	0.031	0.245	0.367	
CLUTCH MEAN	-0.056	0.004	-0.064	-0.049	< 0.001

1

Table 4(on next page)

Phylogenetic Generalized Least Squares scores of variables predicting fecundity in turtles.

Climatic zone 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					0.005
- Temperate	5.104	0.353	4.413	5.796	
- Tropical	5.247	0.503	4.263	6.232	
DIET					0.378
- Carnivore	5.104	0.353	4.413	5.796	
- Herbivore	4.970	0.569	3.856	6.085	
- Omnivore	5.255	0.564	4.150	6.360	
EGG SIZE	-11.426	0.907	-13.203	-9.649	< 0.001

1