The evolution of reproductive strategies in turtles

Gabriel Jorgewich-Cohen[1], Rafael S. Henrique[2], Pedro Henrique Dias[3], Marcelo R. Sanchéz-Villagra[1]

1 Paläontologisches Institut und Museum, Universität Zürich. Zürich, Zürich, Switzerland.

2 Laboratório de Anfíbios, Instituto de Biociências, Universidade de São Paulo. São Paulo, São Paulo, SP, Brazil

3 Departamento de Zoologia, Universidade Federal do Paraná. Curitiba, Paraná, Brazil.

Corresponding Author: Gabriel Jorgewich-Cohen
Karl-Schmid-Strasse 4, Zürich, Zürich, 8006, Switzerland.
Email address: gabriel.jorgewichcohen@pim.uzh.ch

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Abstract

The recorded variation in traits in the egg and clutch among amniotes has led to the idea of an "optimal egg strategy" based on trade-offs, and to hypotheses about general trends. Here we perform an analysis comprising all big clades of living turtles to examine egg and clutch diversity. We include at least one representative of all extant turtle genera. Our goal is to investigate if there are actual trends for reproductive strategies among turtles and to identify factors that influence clutch and egg traits in this amniote clade. Our hypothesis is that turtles have reproductive trends that do not necessarily follow a monophyletic distribution but evolved convergently and in association with specific clades. There are local "optima" correlations between some traits and convergences across phylogeny.

Comentado [x1]: Please, other noun

Comentado [x2]: What do you want to tell as "local"? Are you considering environmental characteristics that can be shaping turtles reproductive strategies?

Introduction

Macroevolutionary researche~~r~~s have focused in many aspects of the diversity of life, including the study of reproductive patterns of amniotes (Battistella et al., 2019; Murray et al., 2020), that can be ~~approached by investigating~~ investigated by ~~the~~ diversity of ~~traits in the~~ egg and clutch characteristics (e.g., Kaplan and Salthe 1979; Deeming and Birchard, 2007; Jetz et al., 2008; Deeming and Ruta, 2014).

The idea of an "optimal" egg/clutch-strategy based on trade-offs, similar to K/r strategies, have led to several yet 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 of phenotypic traits of oviposition strategies are driven by selection, that results in the best adjustments for the production of the biggest number of offspring with the highest fitness and the lowest resource investment by their progenitors as possible (Brockelman, 1975; Congdon and Gibbons, 1987).

Comentado [x3]: What means "strategy" here? You must consider reproductive strategy, egg/clutch size theory, optimal clutch size theory…But, strategy? Please, clarify.

Comentado [x4]: What is this? Reproductive behavior? Nesting behavior?

Turtles offer a rich subject of investigation given the ecological diversity of this group in which all species lay eggs. 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; Rowe, 1994). 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), environmental factors (Macip-Ríos et al., 2013) and/or physiology (Bowden et al., 2004). These hypotheses have been largely based on studies of straight correlations of traits (Gibbons, 1982); methods that take phylogeny into account would provide a test to them.

Comentado [x5]: Why? Please, expand your idea considering evidences for other groups.

Reproductive strategies ~~Available evidence~~ supports the idea that big ~~bodied~~ sized animals tend to produce ~~bigger~~ larger clutches with small and round eggs (Fig. 1A) while smaller species produce small clutches with big and elongated eggs (Fig. 1B)~~, from now on referred as reproductive strategies~~. 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. On the other hand, Pritchard (1979) suggested that small species have a tendency to produce bigger and elongated eggs because a small spherical egg would not be capable of producing a functional hatchling as there is not enough space available and that adult body size is a constraint for the egg width. Moll (1979) argued that spherical eggs are more efficient in occupying limited spaces, therefore larger clutches are supposed to have more spherical eggs.

Although general trends have been identified, a comprehensive phylogenetic analysis ~~comprising all big clades~~ of living turtles in order to explore egg and clutch diversity is still missing. In this study we present ~~several~~ analyzes based on data taken from the literature for at least one representative of all extant turtle genera. Our goal is to examine if there are trends for reproductive strategies among turtles and investigate potential factors that influence clutch and egg traits in this clade. We address the following questions: 1) How do reproductive traits (such as egg size, egg shape, and clutch size) relate to each other within and among turtle clades? 2) How are these reproductive characteristics distributed along turtle phylogeny? 3) Are there "optimal" reproductive strategies? 4) What are the factors that most influence reproductive strategies among turtles? 5) Are there differences on egg/clutch size among turtle families? We hypothesize that although there is variation and ecological factors may affect reproductive traits, these are largely conserved within clades.

Comentado [x6]: Please, check Souza 2004 (https://doi.org/10.11606/issn.2316-9079.v3i1p15-27) and Souza et al 2006 (REPRODUCTIVE ASPECTS OF BRAZILIAN SIDE-NECKED-TURTLES (CHELIDAE; *Bol. Asoc. Herpetol. Esp. (2006) 17 (1)*) for a review in Brazilian Chelidae.

Comentado [x7]: Please, define egg and clutch diversity. Egg size, egg volume, clutch size?

Comentado [x8]: Reproductive strategies is different from reproductive traits.

Comentado [x9]: Reproductive characteristics, reproductive traits, reproductive strategies. Are you using these terms as synonymous?

Comentado [x10]: Which ecological factors? Your introduction did not address this subject.

Materials & 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). All statistical and exploratory analyses were conducted in R 3.2.1 (R Core Team 2016; scripts and input files available on Supplemental Information, Appendix S3).

Exploratory analysis

We performed a principal component analysis (PCA) in order to test the correlations among reproductive parameters (e.g., egg size, egg length, adult body size) commonly tested in previous works with smaller datasets (Elgar and Heaphy, 1989; Iverson, 1992; Iverson et al., 1993; Rowe, 1994). Also, in order to evaluate the impact of phylogenetic relationships over these correlations, we performed a phylogenetic PCA. We used the functions imputePCA (package missMDA; Josse and Husson, 2016) and phyl.pca (package phytools; Ravell, 2012) respectively.

Comentado [x11]: I really do not know how authors performed literature revision but there are some problems with the review showed in S1 and S2. For instance, authors used data from captive Mesoclemmys vanderhaegei (Corazza & Moolina 2004). Captivity is not a good source for data record given several caveats. Souza et al 2006 give some literature in their review (e.g. Cabrera, M.R. 1998. *Las Tortugas Continentales de Sudamérica Austral*. Cordoba, Argentina). The same can be verified for P. geoffroanus (see Souza & Abe 2001. Population structure and reproductive aspects of the freshwater turtle, Phrynops geoffroanus, inhabiting an urban river in southeastern Brazil. Studies on Neotropical Fauna and Environment, 36: 57-62). Most of the habits identified in S1 are nor really true because several species can be found in rivers.

Comentado [x12]: Correlation with what? Auto correlation?

For the phylogenetic PCA, we used a super tree reconstructed in Mesquite v. 3.51 (Maddison & Maddison, 2019), including the same taxa as in the database. We followed the phylogenetic hypothesis proposed by Pereira et al. (2017) for the backbone of our tree—which is the hypothesis with the denser taxonomic sampling available—and positioned 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).

The resulting topology was used to map characters related to reproductive traits using the contMap function of phytools package (Revell, 2012). 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 Fecundity (FEC): maximum number of eggs in a clutch. We also used the phytools package to perform a multivariate analysis using the function phylomorphospace3d, in order to reconstruct the 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) — for turtle phylogeny based on the former proposed characters (ESI, ESH and FEC) as axis. The 3D visualization can be accessed following the R script available on the Supplemental Information (Appendix S3).

Explanatory analysis

In order to address different aspects of reproductive strategies among turtles, we ran two different model selections. In the first one, we used the square root values of the ESI variable as the dependent variable (egg size) to test how the selected independent variables predicted the size of the egg among species (egg size selection). In the second model selection, 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 address how the selected independent variables predicted the fecundity of turtle species (fecundity selection).

For the first model selection, we used a dataset containing at least one representative of each genus with a total of 230 species sampled. For the second model selection, we sampled a total of 177 species; the monotypic genus *Notochelys* (Gray, 1863) was the only one not included due to lack of information on number of clutches per year.

We used maximum likelihood to fit general linear mixed models (GLMMs) with Gaussian distribution for the egg size model selection, with egg size as the dependent variable and built every possible combination of models for the five independent variables, without interactions (Table 1). The final set models of the egg size selection contained 31 candidate models besides the null model. The fecundity model selection was performed using maximum likelihood to fit generalized linear mixed models (GLMMs) with log-normal distribution. We used the fecundity as the dependent variable and built every possible combination of models for the four independent variables, without interactions (Table 1; Clutch Size was not included). The final set of candidate models contained 15 candidate models besides the null model. The information about the family each species belongs to was treated as a random effect in all models (1|family), because preliminary analyses show that closed related species have similar egg size and fecundity (ANOVA $p<0.0001$).

Model selections were performed using the function dredge of the MuMIn package (Bartoń, 2015). We ran an all-subset model selection and ranked the models based on Akaike's Information Criterion corrected for small sample size (AICc; Burnham and Anderson, 2002; Symonds and Moussalli, 2011) with the best-supported model having the lowest AICc.

When there was no single model strongly supported (Akaike weight > 0.9) we calculated the evidence ratio for the best-supported models (Burnham and Anderson 2002; Johnson and

Omland 2004) and used multimodel inference to understand how independent variables predict the reproductive trait in turtles (Burnham and Anderson 2002; Johnson and Omland 2004; Burnham et al. 2011; Symonds and Moussalli 2011). We used all the candidate models for full model averaging and calculated the relative importance of each independent variable and their respective confidence intervals (85%).

Results

Regular and phylogenetic PCAs (Fig. 2A and B) showed similar correlations among inputs. In both cases, there is a positive correlation between egg size characters (length, width and weight), and a negative correlation between egg size and clutch size. The size of adult animals (e.g., carapace length) have little influence over other parameters, with a weak negative correlation with egg weight present only in the regular PCA. Number of clutches per year is the most divergent parameter when comparing both analyzes.

The contMap analysis (Fig. 3A, B and C) allows the easy visualization of characters distribution within the phylogeny. Through the comparison of the plotted trees, it is possible to correlate small and round eggs to species that produce bigger clutches. Such traits evolved independently in several not-directly related families (e.g., Podocnemididae, Cheloniidae, Dermochelyidae, Chelydridae) or even only in bigger bodied representatives in some families (e.g., Testudinidae, Trionychidae). This pattern is also recovered in the phylomorphospace analysis (available on Supplemental Information Fig. S1 on its static view). Species with extreme characters distribution are easily visualized (e.g., *Geoemyda spengleri* and *Glyptemys muhlenbergii* – low FEC, high ESH and ESI; *Lepidochelys olivacea* and *Podocnemis expansa* – high FEC, low ESH and ESI). Although it is possible to visualize some groups of species that fit in "clusters" of extreme character-distribution in the phylomorphospace analysis, most species were positioned close to each other, with average values for all the three characters.

Comentado [x13]: Not so easy to identify. Please, clarify what means the color bar and the similarities/differences among the trees according to the variables.

Comentado [x14]: What does it mean?

Comentado [x15]: Which values?

In the egg size model selection, only the best-ranked model (Clutch Size + Habitat) was selected as a plausible model (ΔAICc < 6; weight = 0.979; Likelihood Ratio Test < 0.001; Table 2). The model predicts that the size of the egg in relation to body size decreases with the increase of the mean number of eggs per clutch, and that aquatic and oceanic species of turtles have smaller eggs than terrestrial and semi-aquatic species, respectively (Fig. 4).

Comentado [x16]: Oceanic and aquatic are both "aquatic"; you are referring as freshwater.

Comentado [x17]: You must identify each plot with a color represented by the habitat

In the fecundity model selection, three of the 16 candidate models were selected as best-supported models (ΔAICc < 6; accumulated weight = 98.6%; Table 3). As the first supported model only accounted for 68% of the variation (Table 3), we used all 16 models for multimodel inference through model averaging to calculate the relative importance of each variable to turtle's fecundity (Table 4). Both habitat and diet were the most important factors to predict turtle fecundity (RI = 1.00). Low relative importance values and confidence intervals including zeros suggest that climatic zone and zoogeography are not good predictors for fecundity.

Comentado [x18]: Where, in the manuscript, you identified these variables? With are the hypothesis and premises for these "variables"?

Discussion

Comentado [x19]: Need deep literature review, including up to date literature.

The evolutionary history of turtles is marked by a complex pattern of character evolution

Comentado [x20]: Show it!

regarding breeding biology. The hypothesis that big bodied turtles tend to produce ~~big~~ larger clutches with comparatively smaller and rounder eggs (group 1) while small bodied species produce small clutches with larger and more elongated eggs (group 2) is supported by the patterns observed in the PCA ~~anlysis~~analysis, by the contMap analysis and by the egg size model selection.

Evolutionary patterns (evidenced by different colors) in all of the three contMap analyzes tend to interact to each other, suggesting a correlation between the tested characters. These patterns evolved independently and recurrently along the diversification of turtles. This shows that during its evolutionary history, turtles explored different reproductive strategies with several instances of convergent evolution.

Although no clear "optimum" reproductive strategy clusters are formed on the phylomorphospace analysis plot, extreme examples of each of the proposed groups are visible (e.g., *Geoemyda spengleri* and *Lepidochelys olivacea*, as representatives of groups 2 and 1, respectively). The difference on scale of character FEC might be partially responsible for the poor visualization, as most species colonized the same portion of the morphospace (bottom of the Y axis). Nevertheless, even discarding this bias, most species would still be clustered in the middle of the plot as they show average values of both ESH and ESI.

One could either argue that there is a third "optimum" cluster of reproductive strategy with average values or that there are no clusters at all. We advocate that there are major trends on turtle reproductive strategies that one could call "optimum constraints", although they seem to be somewhat relaxed as all reproductive characters are continuous, without any clear break in patterns, especially in the case of average values, where most species are clustered.

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 lives in land and Cheloniidae/Dermochelyidae that lives in the ocean, can be considered evidence for adaptation of an "optimal" reproductive strategy at a specific environment or a constrain of specific clades. Although asserting the adaptive value of these traits can be difficult (see Kluge 2005), the fact that the evolution of these strategies is correlated with the colonization of new environments, provides strong support for a heuristic postulation of its adaptive value (Kluge 2005; Losos 2011). Furthermore, our model selection analysis for egg size (Fig. 4) not only corroborates the patterns described by Elgar and Heaphy (1989), but also brings light to the fact that this pattern is not statistically confounded by chelonian families forming ecological groups, since we used "family" as a random factor.

On the model selection analysis, the "semi-aquatic" group presents slightly bigger relative egg size when compared to the terrestrial species that belong to the Testudinidae (Fig. 4; blue and green lines, respectively). Both groups present bigger relative egg size when compared to oceanic and aquatic species (Fig. 4; red and black lines, respectively). This is evidence that "optimal" reproductive strategies are not correlated to species' phylogenetic distribution

Comentado [x21]: Reproductive behavior? Reproductive strategy…?

Comentado [x22]: Again!

Comentado [x23]: How should be this cluster?

Comentado [x24]: ???

Comentado [x25]: Is this a result from evolutionary history or an artefact of the analyses?

Comentado [x26]: Not clear. What really your results show?

Comentado [x27]: You are using theories from 30 years ago, that used different statistical analyses and different approaches, including phylogenetic. Also, you are using "niche theory" to explain your results but did not explained exactly how niche theory can be related with your findings.

Comentado [x28]: Which strategies?

Comentado [x29]: Very confuse. Which are the "patterns" described by Elgar and Heaphy? Which are the similarities and differences of your results and their results? Which are the hypothesis underlying traits such as egg size, egg shape and the habitat (terrestrial, aquatic…)?

Comentado [x30]: ???

(although close related species tend to have similar strategies), but related to their life/reproductive strategies that converged in different clades. The model selection for fecundity indicates habitat and diet as the most influential characters, highlighting the importance of life history traits for reproductive strategy selection.

Comentado [x31]: Explain!

Comentado [x32]: Where did you find information about diet/feeding habits? Your manuscript did not address such life history characteristic as a variable to be be included in the analyses.

Conclusions

Little is known about many aspects of the reproductive behavior within Testudines, and some of them might have a direct correlation with clutch/egg size.~~ 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~~”. Many species of the Testudinidae are known to care for their eggs (Agha et al., 2013), making the safe harbor hypothesis a good explanation for the comparatively big eggs and, maybe consequently, smaller clutches in this family. Other clades of turtles have historically been considered to lack any forms of parental care, but now we have evidence of the opposite (Ferrara et al., 2013). *Podocnemis expansa* is a good example of “group 1” reproductive strategy, being the biggest South American freshwater turtle and producing 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 only weak explanatory power. Other factors probably have bigger influence in this case, such as Elgar and Heaphy’s (1989) proposition that round eggs should suffer less from desiccation.

Comentado [x33]: What? Many species? Parental care in turtles is very rare if all.

Reproductive traits in Testudines evolved independently several times across tree in non-directly related clades, which can be considered an evidence of convergence, and an argument to endorse the existence of adaptive evolution and constraints in reproductive biology, frequently referred as “optimum” reproductive constrains.

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Dr. Richard Vogt provided important literature on egg/clutch diversity. Danilo Muniz and Diogo Melo helped with statistics.

Comentado [x34]: Did you not perform literature search?

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