

Why do birds lay eggs that fail to hatch?

A phylogenetic comparative study on hatchability

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Master's Thesis in Biology
– Biodiversity, Evolution and Ecology

Department of biology
UNIVERSITY OF BERGEN

June 2019

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2019

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Acknowledgements

First, I would like to thank my supervisor, Terje Lislevand, for great help and support through this entire process. Thank you so much for your explanations, corrections and the rewarding discussions we have had. I always felt more inspired and less stressed when leaving your office. Secondly, a huge thanks to co-supervisor Jostein Gohli who was an exceptional help during the analyses and writing process. I am very grateful that you took the time to answer the endless numbers of emails I sent you.

Thank you to my family for your continuous love and support, and thank you for teaching me the value of hard work and persistence. You are the best. Thanks to Andreas for helping me with excel when I thought my dataset was destroyed.

A special thanks to all my good friends for bringing me on new adventures and taking my mind off uni. You have always cheered for me and pushed me onwards, but I love you the most for the amazing memories you keep giving me.

A thousand kisses for my wonderful boyfriend Hans Fredrik – you are my everyday hero!

Thank you so much to the class of 2014, for the good vibes you have kept on bringing through the last five (!) years. I have enjoyed every party, teacher internship and exam period with you guys, #lektorlove. To Vilde Norderval, thank you for the warm hugs, tons of laughs, hikes, swims and lunch breaks we have shared. Your unique initiative will be sorely missed in Bergen. Any student that gets to be taught by you is extremely lucky.

And last but not least, a great thanks to Vilde Aanderaa for teaching me the importance of making the most of every day. Your words, “*vi kan ikke konge livet*”, have been a great reminder through this semester.

Abstract

In birds, an average of 10-15% of eggs reportedly fail to hatch and remain in the nest after completed incubation. This is due to either fertilization failure or embryo death. Hatchability (the proportion of eggs hatching) is known to vary considerably between different bird species, but the explanation for the high average proportion of hatching failure remains unclear. In this study I replicated a previous comparative study on hatchability (Koenig 1982) but in contrast to that study controlled for possible phylogenetic effects. I show that hatchability increases with relative clutch mass (residuals of regression between egg mass and incubator body mass) and absolute latitude, and that open nests show a higher hatchability than closed nests. None of these findings support the prediction that exposure of the eggs to cold temperatures would lead to higher levels of hatching failure. The positive correlation between hatchability and absolute latitude is also negatively affected by the duration of incubation periods. Birds with herbivorous/granivorous diets was found to have significantly lower hatchability than omnivores and invertebrate-eaters, contrary to both my prediction and results of previous studies. Moreover, values of hatchability were generally not more similar between closely related species than between other species. My results demonstrate that hatchability is a complex phenomenon affected by multiple variables. However, the exact mechanisms underlying the observed variation in hatchability are often not clear and need more detailed studies in the future.

Table of Contents

Introduction.....	1
Possible explanations of hatching failure.....	1
Fertilization error	1
Reduced incubation conditions	2
Other causes of embryo death	4
Combination of several causes	4
Previous comparative studies on hatchability.....	4
Importance of considering phylogeny	5
Predictions.....	5
Body mass:.....	5
Incubator:	6
Social organization:	6
Incubation duration:	6
Nest type:	6
Diet:.....	6
Latitude:.....	6
Materials & methods	7
Collecting data	7
Phylogeny	10
PGLS	10
Analyses.....	11
Results.....	14
Discussion.....	16
Incubation limitation.....	16
Latitude.....	17
Nest type	18
Diet.....	19
Factors of uncertainty	20
Phylogenetic signal.....	21
Conclusion	22
References.....	23
Figures.....	28
Tables.....	34

Introduction

In bird nests it is quite common to find abandoned, unhatched eggs left in nests after the chicks have fledged. In fact, different studies report that an average of 10-15% of bird eggs fail to hatch (Koenig, 1982; Morrow, Arnqvist, & Pitcher, 2002; Spottiswoode & Møller, 2003). This is puzzling in evolutionary terms, given the costs involved in egg production for a female bird. "Hatchability" is a term used to describe "*the proportion of eggs hatching of those present in the nest at the end of incubation*" (Larsen, Lislevand, & Byrkjedal, 2003). By this definition eggs that are depredated, accidentally destroyed or abandoned during incubation are excluded from hatchability calculations. Hatchability is known to vary considerably between different bird species, but the explanation for the high average proportion of hatching failure remains unclear. In this master's thesis I aim to test different hypotheses which might explain the observed variation in hatchability. Hopefully this study will contribute to a better understanding of some of the factors that might affect and explain hatching success of birds.

Possible explanations of hatching failure

There may be various proximate reasons for eggs not hatching but all of these could either be categorized as fertilization error or embryo death sometime between fertilization and hatching (Hemmings, West, & Birkhead, 2012). However, most studies on hatchability never distinguish between the different causes of unhatched eggs, but rather just categorizes all of the eggs as "infertile" (Birkhead, Hall, Schut, & Hemmings, 2008). Importantly, and similar to previous comparative work, I am not able to discriminate between these two scenarios of hatching failure in this thesis - since I use data from published studies. Nevertheless, under the hypotheses proposed I do assume that either 1) fertilization error, 2) reduced incubation conditions or 3) other factors leading to embryo death predominate in causing hatching failure.

Fertilization error

Inbreeding, the mating of closely related individuals, can be suggested as a possible cause of infertility in birds. A study of zebra finch showed a higher percentage of abnormal sperm in addition to slower sperm motility, as a result of inbreeding depression (Opatová et al., 2016). Even though the species still had a high percentage of normal sperm with unchanged characteristics, this can possibly lead to unfertilized eggs as it lowers the proportion of

normal sperm in an ejaculate, and might lower the sperm's success in reaching and penetration of the ovum (Opatová et al., 2016).

Cooperative breeding can also be hypothesized as a cause of low hatchability. By having several male birds in one nest there might be a greater interference during egg laying, reducing the chance of successful egg fertilization. Koenig found hatchability to decrease with increasing social organization (1982). However, it is also possible that extra individuals in a nest can help with incubation, thereby possibly contributing to more optimal incubation conditions and consequently higher hatchability.

Reduced incubation conditions

Reduced hatchability could be the result if parents are not able to keep eggs under optimal conditions for embryo development. It is well known that for successful embryonic development to occur, bird eggs of most species must be incubated. Fertilized eggs require specific incubation conditions to hatch successfully, including appropriate temperature and humidity, proper respiratory gas composition, and a regularly turning of eggs (Ar & Sidis, 2002). Parent birds play a significant role in temperature regulation of the eggs by building a suitable nest and then incubating the eggs. It is this bird-nest unit that is crucial for successful incubation (Deeming, 2002b). Birds of different species live in different habitats and build different types of nests. Hence, eggs of different species might experience contrasting environments during embryo development. Nest type can roughly be divided into open and closed nests, and it can be hypothesized that the closed nests shield the eggs more and thereby protect them more against bad weather and high temperature fluctuations than open nests do.

Incubating birds have a brood patch, an area of featherless, vascularised skin on the underside of the bird (Ar & Sidis, 2002). The patch is in contact with the eggs and receives sensory input about the temperature. This information is used to regulate egg temperature by change of blood flow (Ar & Sidis, 2002). The embryo is typically kept at 37-38°C due to the heat supplied by the parent bird (Deeming, 2002b). In cold and temperate climates, the parent is essential in warming the eggs, whereas in warmer climates the bird functions in shading the nest and preventing the eggs from overheating. Due to different climatic conditions it is therefore plausible to assume that proximity to the equator may affect hatchability.

When the incubating bird leaves the nest for short periods during the day, mainly to forage, the egg temperature may increase or decrease depending on the ambient temperature (Ar & Sidis, 2002). This means that the egg does not have a constant temperature but rather varies because of these off-nest periods. The off-nest period is timed by the parent to minimize change in egg temperature, and the attentiveness increases with both colder and warmer temperatures (Ar & Sidis, 2002). Monogamy is a common mating system in birds, and approximately 50% of bird families have shared incubation between parents (Deeming, 2002a). In addition, sole-incubating parents may also be fed by their partner during this period. These types of cooperative organization might limit off-nest periods that would otherwise leave the eggs exposed to ambient climate conditions. By such mutual assistance there is a possibility of the eggs being cared for by a parent at all times.

The incubation-limitation hypothesis (Lack, 1947) proposes that the parents' incubation ability limits clutch size. This clutch size limit might depend on the body size of the incubator. If the total mass of the clutch to be incubated is relatively large compared to the size of the incubator, challenges of covering the eggs may arise and thus lead to sub-optimal incubation (Lislevand & Thomas, 2006). Some studies which have been done with enlarged clutches has shown a reduced hatchability in such clutches compared to control clutches (Andersson, 1976; Arnold, 1999; Engstrand & Bryant, 2002; Larsen et al., 2003; Lengyel, Kiss, & Tracy, 2009; Reid, Monaghan, & Ruxton, 2000), suggesting that a higher clutch mass makes it harder for the incubator to ensure successful hatching of all eggs. The ratio of body size and clutch mass is therefore expected to be of significance to hatchability.

Longer incubation periods increase the risk of time-dependent mortality to eggs (Martin, 2002). Incubation is a crucial period, and if the duration of incubation is long there is a greater risk that something goes wrong with the developing embryo. A positive and strong correlation exists between the weight of bird eggs and the length of incubation (Boersma, 1982), meaning that larger eggs tend to be incubated for a longer time than smaller eggs. This increase in incubation period might also increase the risk of failure. One can therefore hypothesize that species with different incubation duration and egg size will show different values of hatchability.

Other causes of embryo death

Another possible factor that could affect hatchability negatively is pollution. Studies done on the effects of pollutants on bird reproduction show that pesticides, heavy metals and industrial chemicals can lead to lower fertility of eggs, embryo mortality or both. (Dirksen et al., 1995; Eeva & Lehikoinen, 1995; Fry, 1995; Ohlendorf, Hoffman, Saiki, & Aldrich, 1986). For instance, pollutants might accumulate in the egg contents and reach toxic concentration levels (Fry, 1995), thus reducing hatchability. The diverse diets of birds put them on different trophic levels. The higher a bird is in the food chain, the greater the risk of biomagnification – the build-up of chemicals or toxic substances in the body tissues (Kelly, Ikonomou, Blair, Morin, & Gobas, 2007). Top predators such as raptors and sea birds risk exposure to high concentrations of toxins as the pollutants in the tissues of their prey will be accumulated. This can possibly result in lower hatchability of their eggs.

Inbreeding is known to lead to homozygous expression of recessive lethal alleles, and therefore causing the death of the individuals carrying this genotype (Hemmings, Slate, & Birkhead, 2012; Morrow et al., 2002). In small and isolated populations where the range of dispersal is limited and the encounter of related individuals therefore is higher, inbreeding is more likely to occur (Keller & Waller, 2002). This includes populations on islands and small populations of endangered species (Spottiswoode & Møller, 2003). Embryo death might therefore be an expected outcome of inbreeding depression (Hemmings et al., 2012). A study of New Zealand bird species showed that hatching failure was significantly higher in species that had undergone severe bottlenecks (Briskie & Mackintosh, 2004).

Combination of several causes

There is of course also a possibility that hatching failure may be a result of a combination of several causes. For instance, one nest can contain one unfertilized egg, and suboptimal incubation conditions of the same nest can at the same time lead to hatching failure for some of the other eggs of the clutch.

Previous comparative studies on hatchability

A few comparative studies have previously been done on hatchability (Koenig, 1982; Morrow et al., 2002; Spottiswoode & Møller, 2003; Hemmings et al., 2012). The work of Koenig (1982) was the most extensive when considering the number of variables tested and species included. He found that hatchability was explained by latitude, nest type, diet and

sociality. Spottiswoode and Møller (2003) found that high levels of genetic similarity had a negative effect on hatchability, but that this effect was independent of sociality, a contradiction to what Koenig suggested. Morrow et al. (2002) also studied whether polyandry, a form of sociality, affects hatchability, but they did not establish any such relationship. Hemmings et al. (2012) did a study on endangered birds and found that unhatched eggs from captive birds were more often infertile (lacking sperm) than the unhatched eggs of wild populations.

Importance of considering phylogeny

Species have a shared evolutionary history. As a result, they do not represent independent data points, a contradiction to the assumption of many statistical models (Mundry, 2014). For instance, one should expect greater similarity in traits of closely related species than in the traits of more distantly related species, a tendency called the phylogenetic signal. (Keck, Rimet, Bouchez, & Franc, 2016). Consequently, it is of importance to know the phylogenetic relationship between the species of a study. This makes it possible to incorporate this information into the statistical analyses and control for the phylogenetic signal (Symonds & Blomberg, 2014).

The goal of this master's thesis is to replicate Koenig's study on hatchability from 1982. Many of his original hypotheses will be tested again, including the relationship between hatchability and latitude, diet, nest type, clutch size, number of incubators and social organization. Additional hypotheses of incubation limitation and incubation duration are also included. I also explore if there are differences in hatchability between the species distributed in different fauna-geographical regions. However, one main difference between my study and the study of Koenig is the phylogenetic component. Koenig did not consider nor control for the common evolutionary history of the avian species of the dataset. It is possible that different results would have been generated if this was done, which is one of the questions the current study aims to answer.

Predictions

Body mass:

From the assumption that body size of the incubating birds relative to the total size of the clutch determines incubation conditions, I predict that the risk of hatching failure increases with relative clutch mass.

Incubator:

Due to the possibility of continuous parental care I expect higher hatchability in bird species sharing incubation than in the species where there is only one sole incubator.

Social organization:

From the findings of Koenig (1982), I predict the highest hatchability in monogamous species and lowest hatchability in cooperative breeders.

Incubation duration:

Following the assumption that longer incubation periods are associated with a higher cumulative risk of hatching failure I predict higher hatchability in species with shorter incubation periods.

Nest type:

Higher hatchability in closed nests than in open nests can be predicted on the assumption of more protected incubation in enclosed nests.

Diet:

I expect vertebrate-consuming species to experience lower hatchability than birds with other diets, based on the risk of biomagnification in high trophic levels.

Latitude:

The cold of the poles and the heat of the equator propose quite opposing incubational challenges. I predict that hatchability either decreases or increases with increasing latitude, depending on which challenge is harder to overcome.

Materials & methods

Collecting data

I collected data from primarily three comparative studies to build a relatively large dataset on hatchability. These studies were from Spottiswoode & Møller (2004), Morrow et al. (2002) and Møller, Erritzøe, & Rózsa (2010). Two of the datasets were found in appendices online, whilst one was accessed by contacting the authors. A few additional species was added from Hansen, Schmidt, & Reneerkens (2011), from Croxall, Rothery, & Crisp (1992) and the nest card archive at The University Museum of Bergen. Some of the datasets had an overlap in species. Usually, the reported hatchability was identical in these cases and the references matched. In the events where two of the datasets included the same species but had different references and values of hatchability, a mean value was calculated. The search resulted in 174 individual species in total (see electronic Appendix A).

Koenig's definition of hatchability is the basis of this study. The abovementioned comparative studies consistently used the term "hatching success", not "hatchability". To ensure that the terms had the same meaning and that eggs lost to predation, abandonment or accidents were excluded from the calculation, each study's definition of 'hatching success' was checked and approved before using the data. Other studies were rejected because their definitions were absent or not suitable.

The dataset "*Avian body sizes in relation to fecundity, mating system, display behavior, and resource sharing*" (Lislevand, Figuerola, & Székely, 2007) was used for data on male and female body weight, clutch size and egg mass. For species where information could not be retrieved from Lislevand et al., the online version of Handbook of the Birds of the World (HBW Alive, 2019) was searched for each species. Where only ranges were reported for egg mass, body mass and clutch size I used the midpoint.

Social organization in the study of Koenig (1982) was divided into the categories 1) monogamy 2) polyandry and 3) cooperative breeder. Lislevand et al., (2007) provided information about mating system using the following groups: 1) polyandry; 2) monogamy (<5% polygyny); 3) mostly monogamy, but occasional polygyny (5–15% polygyny); 4) mostly polygyny (> 15% polygyny) and 5) lek or promiscuous. These groups were merged to better fit the categorizations of Koenig. Group 2 and 3 represent monogamy, group 4 and 5

polygamy and group 5 (one species) was categorized as “other”. To obtain the category “cooperative breeding” Emlen and Vehrencamp’s definition, in Arnold & Owens (1998), was used. They define cooperative breeding as situations where “*more than two individuals rear the chicks at one nest*”. A list of such species is given by Brown (1987). The reference “Birds of North America” (Birds of North America, 2019) also report cooperative breeding as a category in their species accounts, which helped to register some of the North American species of the dataset. Additional species was added from the dataset of Spottiswoode and Møller (2003) which also contained this category. These three references made the basis for which species that were considered to be cooperative.

In the datasets where the reference for hatchability was available, I could usually find the individual studies’ latitude-coordinates in the method-section of each paper. In cases where the reference was missing, where the reference was unavailable or where the research papers did not specify the coordinates, I used the species-specific distribution maps in the Handbook of the Birds of the World (HBW Alive, 2019). By using google maps and registering the northernmost and southernmost distribution of a given species, a latitudinal midpoint was determined by calculating the mean value of the two. Only the year-round and breeding distribution was considered when calculating the midpoint-distribution. In a few studies the methods section was inaccessible, but the research area was stated in the title. In those situations, the latitude was obtained from google maps. In the case of Smith's longspur, the research site was only assumed based on knowledge of the researcher’s previous work with this particular species. All registered latitudes were converted to absolute values to represent the distance from equator.

In addition to latitude, every species’ fauna-geographical breeding distribution was also registered. The Palearctic (Europe, northern Africa, North Asia and parts of the Middle East), Nearctic (North America and Greenland), Neotropical (South America), Afrotropical (southern Africa), Oriental (South, East and Southeast Asia), Australian and Antarctic were the seven different geographical zones used (Encyclopaedia Britannica, 2019). Many species have a broader distribution than exclusively one of these zones and was therefore registered to several areas.

I extracted information about the number of incubators and incubation duration for each species using the Handbook of the Birds of the World (HBW Alive, 2019). For North American

species Birds of North America (Birds of North America, 2019) was searched instead. Incubator was divided into the categories “uniparental” and “biparental”, depending on whether the female/male was the sole incubator or if there was a shared incubation. When both sexes shared incubation I made no distinction between how much each parent contributed. For a few species there were reports that male birds could help with incubation under extreme weather conditions, but the species were still classified as “uniparental” in these cases. Incubation duration was defined as the number of days between egg laying and hatching that a species actively incubated their eggs. This information was usually stated as an interval, so the midpoint was used as a measure for the number of days of incubation. When different studies were referred to, the mean of their results was used.

Sibly et al. (2012) provided a dataset of nest type, including the categories “open”, “closed”, “cave” and “cavity”. I only wanted to distinguish between open and closed nests, considering exposure to the weather. Cavity was defined as a nest “placed in a cavity with a narrow/protected entrance”, whereas cave was a nest “placed in sheltered locations that are not true cavities”. “Cavity” and “cave” was therefore recategorized to both be included in “closed”. The Handbook of the Birds of the World (HBW Alive, 2019) was used to get information on the species that were missing in the dataset of Sibly et al (2012). Nests described as cups or shallow scrapes were registered as open, whereas descriptions of tightly woven and domed-shaped nests were categorized as closed, as well as nests in burrows and hollows.

Sibly et al. (2012) also provided data on the diet of most species in my dataset. The diet groups I used was “Primarily granivorous/herbivorous” (which included “seed”, “nectar”, “fruit” and “vegetation”), “Invertebrates”, “Omnivore” and “Vertebrates” (equivalent to VertFishScav from Sibley et al., defined as birds that «preys upon or scavenges vertebrates»). Remaining species’ diets were found in Handbook of the Birds of the World (HBW Alive, 2019). In species where diet varies between seasons the diet during breeding season was the one of interest to this study.

Three more variables were added to the dataset by combining some of the other variables collected. By multiplying the egg mass with the clutch size, I got a variable representing the total clutch mass of each species. A variable called “weight of incubating parent” was also constructed by using data on incubator, female body weight and male body weight. In the

cases with biparental incubation the mean parent weight was calculated and used, and for uniparental cases the weight of the reported incubating sex was used.

Phylogeny

The analysis I wanted to do should control for the shared phylogeny between the 174 species of the dataset. Therefore, I needed to construct a phylogenetic tree to represent this relationship. To do this the Global Phylogeny of Birds (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012) was used. The website www.birdtree.org is based on this phylogeny and provides a tool for producing phylogenetic trees using your own subset of taxa. By providing a list of Birdtree-accepted species names one receives a file with a set of different trees made from this information. The trees received are based on either the backbone phylogeny of Ericson et al. (2006) or Hackett et al. (2008) depending on the settings (Rubolini, Liker, Garamszegi, Møller, & Saino, 2015). The Hackett sequenced species were used in this case. By using the program TreeAnnotator the information in the trees from Birdtree are summarized and a single consensus tree chosen. This tree has been selected on the basis of which tree structure that was the most common of the different trees provided, as well as the posterior probability of each node, meaning the frequency of a particular node in the set of trees. The consensus tree was incorporated as a phylogenetic hypothesis in the later statistical models (Rubolini et al., 2015).

There are about 10 000 known extant bird species in the world, but the backbone phylogenies of Hackett and Ericson are based on genetic data from approximately 6670 of these (Rubolini et al., 2015). As a result, not all the species in my dataset could be included in the phylogenetic tree. Genetic data on *Turdoides squamiceps*, *Nectarinia osea*, *Circus pygargus* and *Gallinula mortierii* was unfortunately missing and these species therefore left out from further analyses, reducing the number of species to 170.

FigTree is a program exclusively made for viewing phylogenetic trees (Beast Developers, 2017). When a consensus tree has been chosen in TreeAnnotator one can view it and adjust the layout using FigTree. Figure 1 shows the consensus tree that was produced for the current study's analyses.

PGLS

The analysis used here is phylogenetic generalized least squares (PGLS). This is a type of linear regression where information about phylogeny is used to reduce the variance around

the estimated regression line (Gonzalez-Voyer, 2018). Because of the shared ancestry of the bird species, there is an expected covariance among the residuals, which is expected to be high in closely related species, low in distantly related species and absent in unrelated species (Symonds & Blomberg, 2014). The phylogeny obtained from TreeAnnotator will in this study be used to calculate the expected covariance structure in the data. This information is then applied to the generalized least squares regression equation to correct for phylogeny to the appropriate degree (Symonds & Blomberg, 2014). The strength of the phylogenetic signal is denoted by the symbol lambda (λ) and takes a value between 0 (no phylogenetic signal in a trait, i.e. no tendency that closely related species are more similar) and 1 (a strong phylogenetic signal where there is a clear tendency of similarity between related species) (Symonds & Blomberg, 2014).

Analyses

I used the software R (version 3.4.3) to conduct the analyses of the study (R Core Team, 2017). The relevant packages used was ape, caper, phytools, geiger and interplot. The analyses started with a test of phylogenetic signal in the trait hatchability itself, testing the hypothesis that related species resemble each other more in their values of hatchability.

Through a look at the response variable hatchability it became clear that there was a distinct outlier in the dataset. This was the Galapagos Hawk with a hatchability of only 45% %, which is much lower than the second lowest value in the data set (61% in the 169 species).

Univariate PGLS-analyses were performed with hatchability as the response variable, both with and without this outlier. The outlier was found to create a significant association with clutch size that was not replicated when the outlier was removed. The outlier was therefore dropped from the analyses, making the number of included species 169. In the univariate analyses with categorical predictors a one-way ANOVA was used to obtain comparisons between the different levels. To get comparisons between all levels and uncover any significant differences, the order of the levels in a factor was shuffled.

One univariate linear regression analysis was also done with clutch mass as the dependent variable and body mass of incubating parent as the independent variable. The resulting residuals of this regression were saved a new predictor variable (residual clutch mass) to

represent the relative relationship between mass of clutch and parent. Residual clutch mass was then included in an analysis with hatchability as the response variable.

A given dataset can be described by many potential models. My goal was to find the model that has the best fit to the data and high explanatory power (Crawley, 2007, p.324). To obtain this, I performed a backward stepwise model selection, starting with the maximum model with all possible explanatory variables included. However, before doing this, potential correlations between the continuous variables had to be detected. One of the assumptions of a multiple linear regression is that the independent variables cannot be too highly correlated (Crawley, 2007, p. 448). Before the model selection could start, I checked for correlation among the continuous predictors by using Pearson's correlation coefficient (Table 1). A correlation coefficient of above 0.6 or below -0.6 was set as a limit for deciding correlation. The univariate models of the predictors that were correlated were compared using Akaike information criterion (AIC). The model with the highest AIC-value was considered to have lower explanatory power, and the predictor was therefore removed before further analyses. One predictor was left out from the model selection because it was strongly correlated with another predictor (Table 2). In addition, four other variables were left out due to missing data for a high number of species (Table 2). Inclusion of these variables would have reduced the dataset to only 104 species and was therefore avoided.

The model selection thereby started out with latitude, diet, nest type and incubation duration as the predictor variables. I performed a PGLS-analysis on this maximum-model, and the predictor with the highest p-value was removed to make a simpler model. These two different models were then compared using AIC, which estimates the quality of a model while also penalizing the number of parameters included (Field, Miles, & Field, 2012). This process of predictor-removal and AIC-comparison was repeated until no further predictor could be removed without giving a model with higher AIC and statistically significant loss of fit. The maximum-model and this new simplified model were both tested for significant interactions between predictor variables. Model selection was then repeated for a second round, now with the discovered interactions included in the simplified model. The resulting model is the minimal adequate model to describe hatchability given the dataset.

To control that both the univariate models and the minimal adequate model works well for the data, I used diagnostic plots in R to examine that the residuals of the models had linear

patterns and were normally distributed (Kim, 2015). This tells us that we have modelled a linear relationship, and that the differences between the observed value and the model are close to zero (Field et al., 2012, p.272). Some of the models had some distributional problems which was improved after log-transforming the response variable “hatchability”. This does not change the relationship between variables, only the units of measurements (Field et al., 2012, p.191).

The significance level (alpha) of the analyses was set to 0.05. Means are presented +/- standard deviation.

Results

The mean hatchability of the 169 species of the sorted dataset was 90,0% ($\pm 7,05$). The range varied from 61% in *Loxioides bailleui* to a reported 100% in the three species *Acrocephalus taiti*, *Calidris maritima* and *Promerops cafer*. The frequency distribution of hatchability is presented in figure 2, showing that the majority of the observations is found around the median of 91,3. There is also a large variance within the continuous predictors (Table 3).

The test of phylogenetic signal in the response variable hatchability showed that $\lambda=0$, equivalent to no phylogenetic signal. Hence, there was no tendency that more closely related species had more similar values of hatchability than more distantly related species. Despite a missing phylogenetic signal in this trait alone, there is a phylogenetic signal when hatchability is regressed against both the mass of the incubating parent ($\lambda=0,67$), the number of incubators ($\lambda=0,71$), latitude ($\lambda=0,52$) and incubation duration ($\lambda=0,63$) (Table 4).

The univariate PGLS-analyses with hatchability as response variable are presented in table 4. The significant predictors of the tests were latitude, diet, nest type and residual clutch mass. Latitude was highly significant ($P<0,001$) showing that hatchability increases with increasing absolute latitude (Figure 3). Nest type ($P<0,05$) shows a higher hatchability in open nests compared to closed nests (Figure 4). Figure 5 illustrates how hatchability varies between bird groups with different diets ($P<0,05$), with herbivorous/granivorous species having the lowest hatchability. The one-way ANOVA showed that herbivorous/granivorous species are significantly different from invertebrate-eating species ($P<0,01$) and omnivores ($P<0,05$), and that there were no significant differences between the remaining levels. The regression analysis of clutch mass and weight of incubating parent was also highly significant ($P<0,001$), with clutch mass increasing along with the weight of the incubator (Figure 6). When the resulting clutch mass residuals were plotted against hatchability this regression was positive and significant too ($P<0,001$) (Table 4a+b). This regression is shown in figure 7.

Regarding the different fauna-geographical regions, the species from two of the zones were significantly different from the rest of the world. The birds in the Neotropical zone had a lower hatchability ($P<0,01$) compared to species that do not occur in this part of the world (Figure 8). Birds of the Palaearctic on the other hand had a higher hatchability than the birds outside this zone ($P<0,001$) (Figure 9).

The minimal adequate model showed that hatchability was explained by diet and an interaction between latitude and incubation duration (Table 5). For every one-unit increase in one of these two interacting variables the slope of the model line of the other variable will decrease with -8.11×10^{-3} (Figure 10). Adjusted R^2 for the minimal adequate model was 0.12, meaning that approximately 12% of the variance in hatchability was explained by the model. The phylogenetic signal of the model is 0.

Discussion

I found several variables to significantly explain the variation in hatchability in birds. Some of my findings support the conclusions of Koenig (1982). The fact that both studies have made similar discoveries suggest that these types of comparative analyses are helpful in providing a better understanding of the underlying challenges of reproduction in birds. However, some of the relationships I discovered went in the opposite direction of predicted and some do not concur with those of Koenig (1982). Several key findings of my study show that there is little support for the hypothesis that low temperatures could explain the variation in hatchability in birds. These findings are: 1) hatchability increased with relative clutch mass 2) hatchability increased with absolute latitude, 3) hatchability is significantly higher in the Palaearctic region compared to the average for the rest of the world, 4) that the number of incubators had no effect on hatchability, thus suggesting that a continuous incubation is not essential, and 5) the fact that open nests show no sign of lower hatchability despite the fact that egg are more exposed than in closed nests. In the following I discuss these issues more thoroughly.

Incubation limitation

There was a positive correlation between hatchability and the residual clutch mass (Figure 7). This was a surprising result since one should expect that a clutch mass that is relatively small compared to the body mass of the incubator would experience higher hatchability as the incubator would be able to provide extensive coverage and regulation of the eggs. The contrary was found to be true – hatchability increases as the relative clutch mass goes up.

A clutch mass may be heavy due to a large number of eggs. Several studies show that eggs in enlarged clutches warm each other, leading to slower cooling when the clutch is left unattended (Boulton & Cassey, 2012; Reid et al., 2000). Contrary to predicted by the incubation limitation hypothesis, the expected constraint on how many eggs a parent bird can incubate simultaneously, there might be a selection pressure of laying larger clutches in cold climates to prevent cooling (Reid et al., 2000). By doing this, the incubator can leave the nest to forage, and the eggs will experience a higher mean temperature than if the clutch was smaller. By regressing absolute latitude against residual clutch mass I found a positive correlation in my material ($p=0.014$, adjusted $R^2=0.04$, $\lambda=0.94$; own unpublished results). Hence, this supports the hypothesis of Reid et al.

Another possible explanation for why birds may lay heavier clutches than expected from their body size is that a heavy clutch is a larger investment. If there is a low risk of hatching failure, females may lay heavy clutches to ensure high reproductive success. On the contrary, if there is a high risk of hatching failure, a lower clutch mass can be expected. In these latter situations, there is an extensive possibility that the eggs will never result in hatchlings. Hence, it will not be beneficial to invest a lot, given that the chance of success is low. All conditions that may affect the risk of hatching failure are not known but might be related to the factors of significance to hatchability that was revealed through the analyses, for instance latitude.

Latitude

Strengthening the finding of Koenig (1982) hatchability was shown to increase with increasing latitude. Supporting this result is the finding that species of Palaearctic and Neotropical regions have respectively higher and lower hatchability of species in other parts of the world. From this it seems that the progressively colder climate towards the poles is not problematic to deal with for incubating birds, at least not in the sense that it leads to a lower hatchability. Instead, it is possible that hatchability results from a higher risk of overheating in the tropics. Studies done by Webb (1987) concluded that embryos are more susceptible to die from overheating than from getting too cold. In fact, studies of egg cooling in different avian species have shown eggs that hatch successfully after several hours of exposure to temperatures between 10°C and near freezing (Webb, 1987). When the ambient temperature of the nest is high, however, there is an increased challenge for incubating birds to keep their eggs cooled, and especially during off-nest periods there is a risk of eggs overheating (Ar & Sidis, 2002). All avian species have an upper lethal temperature (ULT) which will result in embryo mortality if reached or exceeded (Reyna & Burggren, 2012). The tolerance of high (and low) temperatures is dependent on both exposure time, the rate of heating and the embryonic age (Webb, 1987). ULT might vary slightly between different species, but standard reports are between 42-45°C (Reyna & Burggren, 2012). Such summer temperatures are not unusual at lower latitudes. Nevertheless, other explanations could not be ruled out and further studies on this issue are warranted.

There was an interaction between latitude and incubation duration on hatchability in my analyses. This finding is somewhat complicating the results of Koenig (1982). Rather than

viewing how latitude affects hatchability alone, one must consider how incubation duration impacts this relationship. As figure 10 illustrates, the positive effect of absolute latitude on hatchability decreases with increasing duration of incubation. In other words, the absolute latitude's effect on hatchability is stronger when the incubation period is reduced. Hence, the positive relationship between hatchability and latitude is weakened for species with longer incubation. A possible explanation is that when incubation duration is long the bird will have to start incubation earlier in the season, at a time where colder conditions may be a greater constraint on successful incubation. The incubator will also have to endure and keep the eggs sufficiently warm for a longer period. This is the only result of the study suggesting that cold environment affect hatchability, and it is only in relation to incubation duration. Nevertheless, other explanations for the interaction could not be ruled out and more studies are needed to elucidate causes and effects in the relationships between hatchability, latitude and duration of the incubation period.

Nest type

Closed nests were shown to have higher hatchability than open nests (Figure 4), contrary to what I predicted if hatchability was related to ambient temperatures, but consistent with Koenig's (1982) results. One possible explanation for this finding might perhaps be differences in microbial flora between nests. Microbes are known to penetrate egg shells and infecting eggs (Cook, Beissinger, Toranzos, Rodriguez, & Arendt, 2003), and are associated with embryo mortality (Baggott & Graeme-Cook, 2002; C. M. Hansen, Meixell, Van Hemert, Hare, & Hueffer, 2015). Especially prior to incubation, before the antimicrobial enzymes of the albumen are exposed to optimal temperatures, the eggs are more susceptible to infection (Cook et al., 2003). Cavity-nesting birds are known to reuse nest sites (Aitken, Wiebe, & Martin, 2002), and it is possible that leftover excreta, fungi and other bacterial sources from previous hatching seasons might accumulate in nest sites over time (Wang, Firestone, & Beissinger, 2011). Previously used bird nest boxes have been found to have higher microbial loads on eggs than eggs in new boxes (Wang et al., 2011), and also fungi overwinter in previously used cavities (Baggott & Graeme-Cook, 2002). Microbes thrive under humid conditions as it facilitates both microbial growth and penetration through the pores of the egg (Cook et al., 2003). According to Baggott & Graeme-Cook (2002) humidity is expected to be higher in cavity nests than open nests. But considering the exposed design of open nests it is quite likely that humidity will also be high in open nests in areas of regular

rainfall. A study on cup and cavity nests of domestic chicken eggs found that microbial growth was higher in open nests (Godard, Wilson, Frick, Siegel, & Bowers, 2007). Moreover, my definition of closed nests also includes other types of closed nests than cavities, for instance dome-shaped nests built new each year. In such nests there is no expectation of any microbial differences from open nests. It is therefore not likely that microbial fauna is the only factor explaining the differences in hatchability between open and closed nests. Humidity is therefore no clear cause of the observed variations.

It could be criticized that my definitions of “open” and “closed” nests are too wide. By such simplifications one may lose interesting aspects of the variation in the data, and typically end up with no significant findings. Nevertheless, in this study I found species with open nests to have significantly higher hatchability than those with closed nests. Even though no obvious explanation can be proposed, it is noteworthy that Koenig found the same results in 1982. He speculated that inexperienced birds have lower hatchability and are more exposed to predation of nests. If this is true, then amongst the open nesting birds, nests of inexperienced birds might be lost to predation more frequently. The remaining nests, representing a high frequency of experienced birds, are included in the hatchability-calculations and would therefore result in relatively high values of hatchability. Closed nests on the other hand are more protected against predators, possibly leading to a higher survival of nests of inexperienced birds in this group than in the open nest group. These will be included in the calculations and hence pull the mean hatchability down. It will therefore appear as if the closed nests have a lower hatchability than open nests. To my knowledge, this hypothesis has not been studied further. Additional studies on natural built nests and wild birds are needed to obtain a greater understanding of nest type and hatchability.

Diet

The result that species with a primarily herbivorous/granivorous diet had significantly lower hatchability than species of both omnivores and invertebrate-eaters was unexpected. Herbivores/granivores are primary consumers on low trophic levels and would therefore not suffer from the accumulation of contaminants to the same degree as species on higher levels. If herbivorous/granivorous species spent longer time foraging for food or regularly experienced nutrients-deficiency from their diet this could be a possible explanation for the observed low hatchabilities, but I found no research claiming this. However, contrary to my

findings, in Koenig's test of diet the herbivores/granivores had the highest values of hatchability, significantly higher than primary carnivores/insectivores and secondary carnivores. These results thereby support the hypothesis of more negative effects of pollution in higher trophic levels. Still, there was no difference between the two carnivorous group, differing from my prediction again. Because of two contradicting results from two different comparative studies there seems to be a need for additional research to reveal the actual relationship of diet and hatchability.

It should be noted that it could also be useful to increase the sample sizes of several of the different diet categories from both studies. While 100 species of my dataset ate invertebrates, the sample size of respectively herbivores/granivores, omnivores and vertebrate-eaters was 34, 16 and 19. Likewise, Koenig's group of secondary carnivores only included 17 species, compared to $n=53$ and $n=85$ for the other groups. These groups between 16-34 species are quite small and can possibly prevent a representative hatchability-estimation of each group. By including more species of these diets before repeating the analysis, different results might be yielded.

Factors of uncertainty

One possible reason that different results was sometimes obtained from my study and the study of Koenig (1982) may be that the dataset of Koenig is not published online and could therefore not be acquired. Thus, even though there is probably an overlap, different species and/or data may be used in the analyses of the two studies. Further, the adjusted R^2 of the model tells us that it explains about 12% of the variation in hatchability. This means that approximately 88% of the variation remains unexplained. There are at least two possible factors that might have added uncertainty and noise to my data set to such degree that analyzed relationships got a low explanatory value or even remained undetected in my analyses. First, since I have used data from various sources, I cannot rule out the possibility that some of the data contain errors and uncertain data points. Second, an optimal study on hatchability would distinguish between unhatched eggs resulting from infertility, and unhatched eggs resulting from embryo death, and test separate hypotheses concerning each case. One weakness of this study is that we do not know if the unhatched eggs of the dataset are due to infertility or embryo mortality since the studies used do not discriminate between these two causes. It is probable that a mixture of both types of hatching failure is

represented. However, in most of the predictions made, fertilisation of the eggs is an underlying assumption, for instance regarding the incubation ability of the parent. If the egg is not fertilised and hence does not contain an embryo, the heat and coverage provided by the incubator is irrelevant as the egg will never hatch anyway. If many of the eggs reported are unfertilized, this could explain the absence of expected results. Future studies should decide if eggs are unfertilized or not in order to understand the underlying explanations of hatching failure. However, if the data was not good, I would not expect to find any significant relationships, and it would be difficult to find support for the results of Koenig (1982). The fact that such results actually are obtained indicate that the results are solid and that the variation in hatchability is not random.

Phylogenetic signal

Slowly evolving traits have been identified as phylogenetically constrained (Blomberg, Garland, & Ives, 2003). An evolutionary constraint means that the course or outcome of evolution is somehow limited (Hansen, 2015), and will keep a trait from evolving quickly, thereby maintaining a high similarity between related species. These constraints can thus lead to a phylogenetic signal. On the other hand, some traits appear to be evolutionary labile, changing easily. In such traits related species are not necessarily more similar (Symonds & Blomberg, 2014), and thus lacking a phylogenetic signal. In my analysis, the trait hatchability showed no phylogenetic signal. This could mean that hatchability is not constrained, but rather labile, likely driven by ecological and abiotic factors.

It can be discussed whether the results of the work of Koenig are valid even without inclusion of phylogeny. A phylogenetic signal was found in several of my univariate models. If phylogeny was not considered here, a less correct regression would have been made because this signal would not have been controlled for. However, in the minimal adequate model there was no phylogenetic signal. This means that an analysis could have been performed without the use of PGLS, and the results would be the same as for an ordinary least squares regression model (Symonds & Blomberg, 2014). Due to the consistency of the results between mine and Koenig's studies (1982) it would seem like the validity of his studies are quite high. Nevertheless, there is no disadvantage of using phylogeny in a study like these, as the PGLS will not over-correct for phylogeny, but rather correct to the appropriate degree (Symonds & Blomberg, 2014). The possible consequences of ignoring

phylogeny, on the other hand, is serious. A study from 2002 reviewed 26 phylogenetic comparative data sets. Of 103 studied traits 60% showed phylogenetic associations (Freckleton, Harvey, & Pagel, 2002). The study concluded that a better model of the variance in the data was provided when including phylogeny, and suggested a consistent use of λ when analyzing comparative data (Freckleton et al., 2002).

Conclusion

To conclude, hatchability is a complex phenomenon affected by multiple variables. This study shows that phylogenetic comparative studies are helpful in investigating and explaining the variation in hatchability. However, even though several significant relationships have been revealed through my study, there are underlying mechanisms of hatchability that are not yet understood, for instance in relation to nest type, residual clutch mass and the interaction between latitude and incubation duration. This calls for increased attention to the field and additional research. Such future studies should also try to distinguish between the causes of hatching failure and test distinct hypotheses of each cause.

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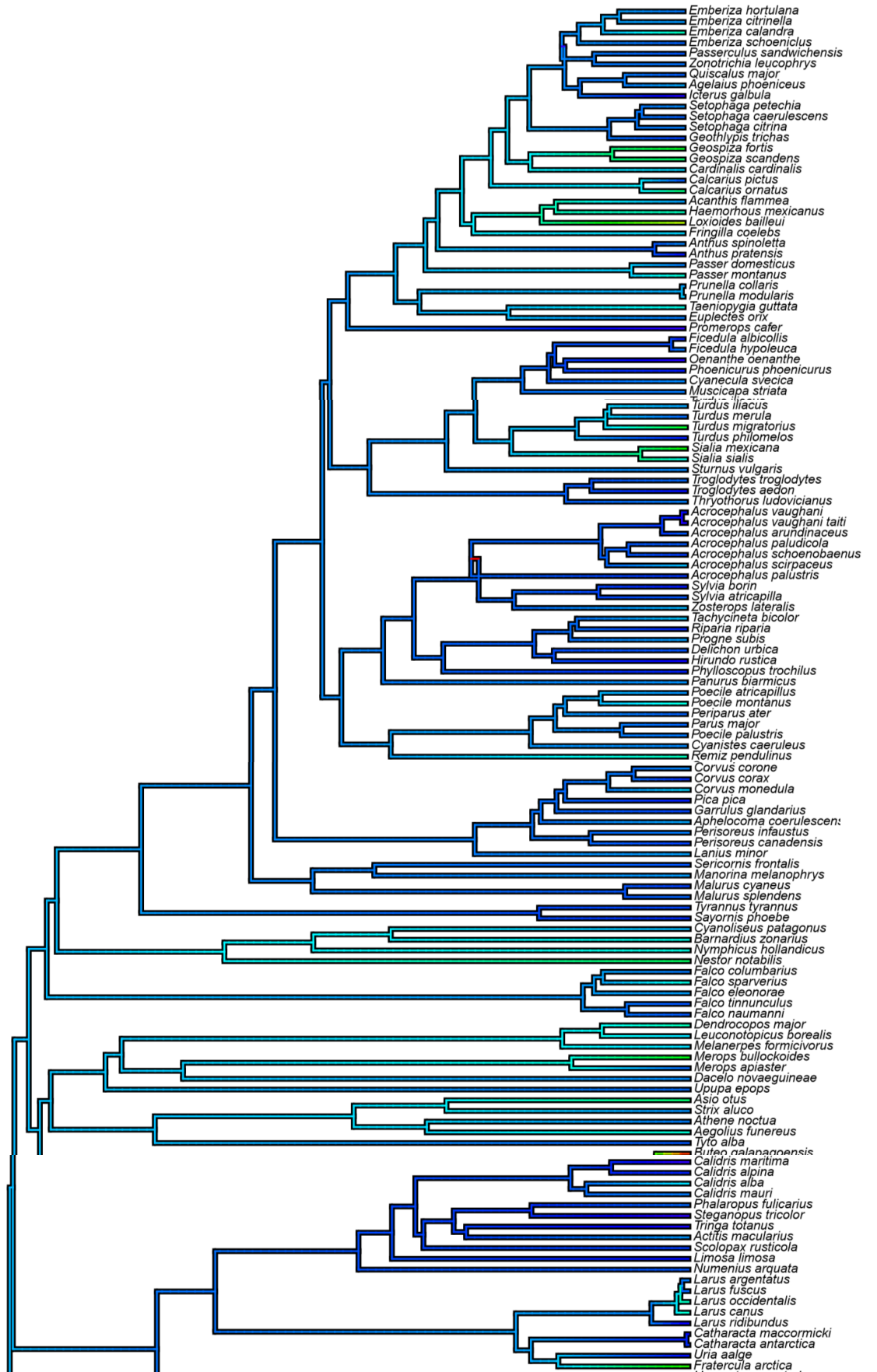
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Figures



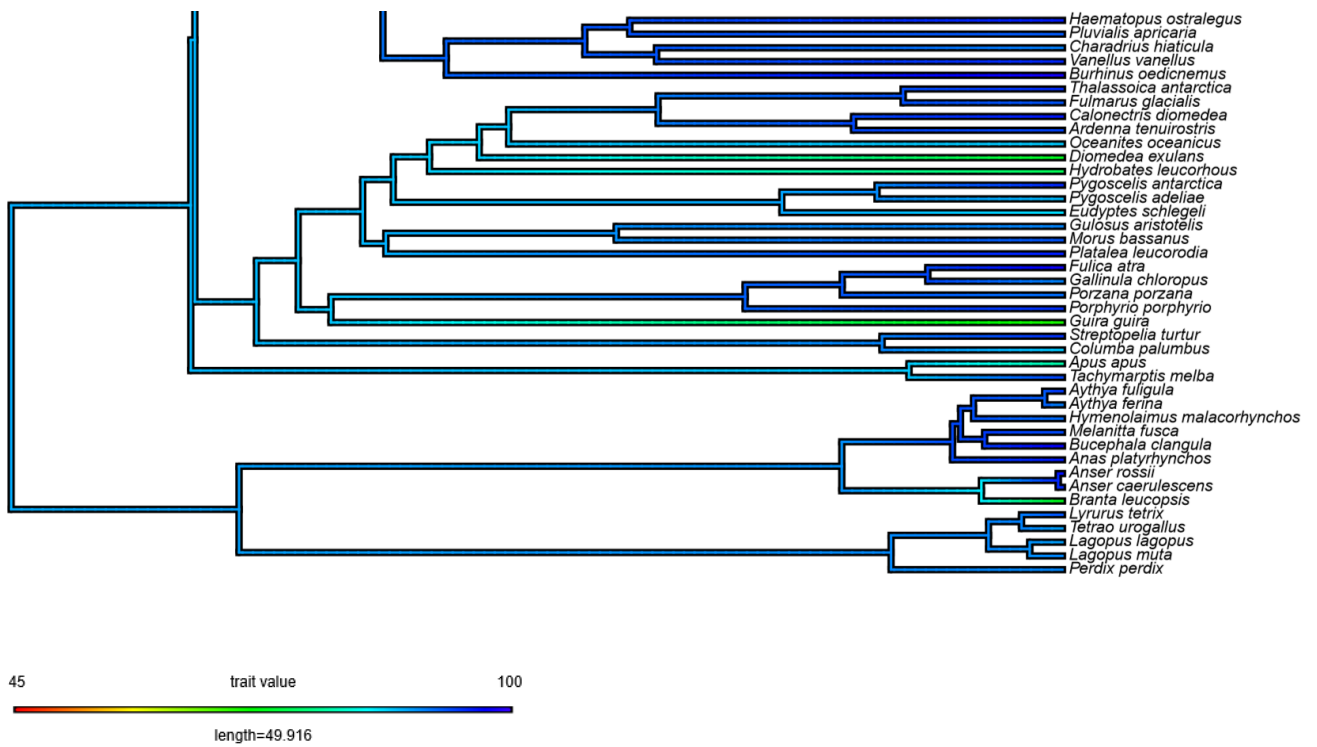


Figure 1: The phylogenetic tree constructed from TreeAnnotator, including the 169 species of the analyses.

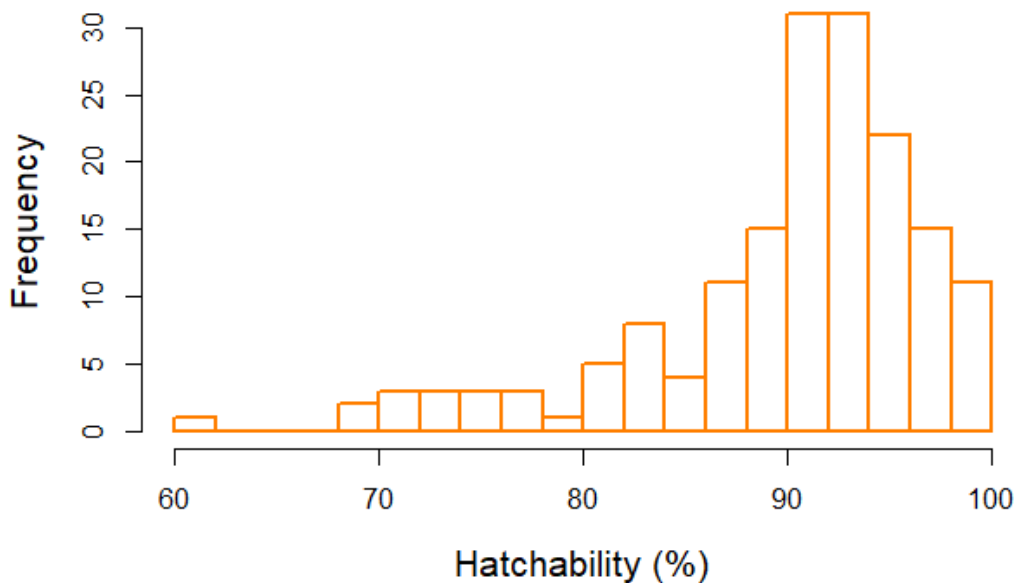


Figure 2: The histogram shows the frequency distribution of hatchability for the 169 species of the analyses. Hatchability ranges from 61-100%, with values of 91-94% having the highest frequencies.

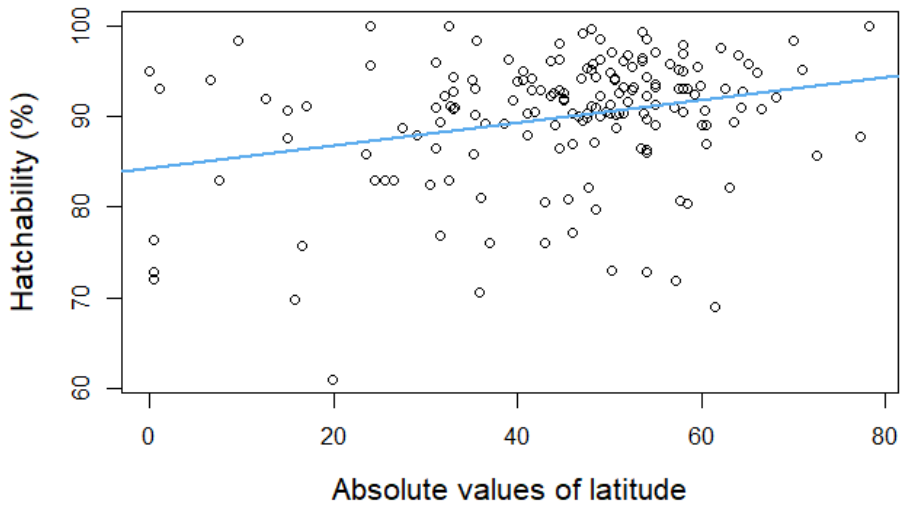


Figure 3: Regression of hatchability against latitude. Hatchability increases along with absolute latitude, meaning that the values of hatchability gets higher when moving towards the poles. The linear expression of the regression line is $y = 0.12x + 84.4$.

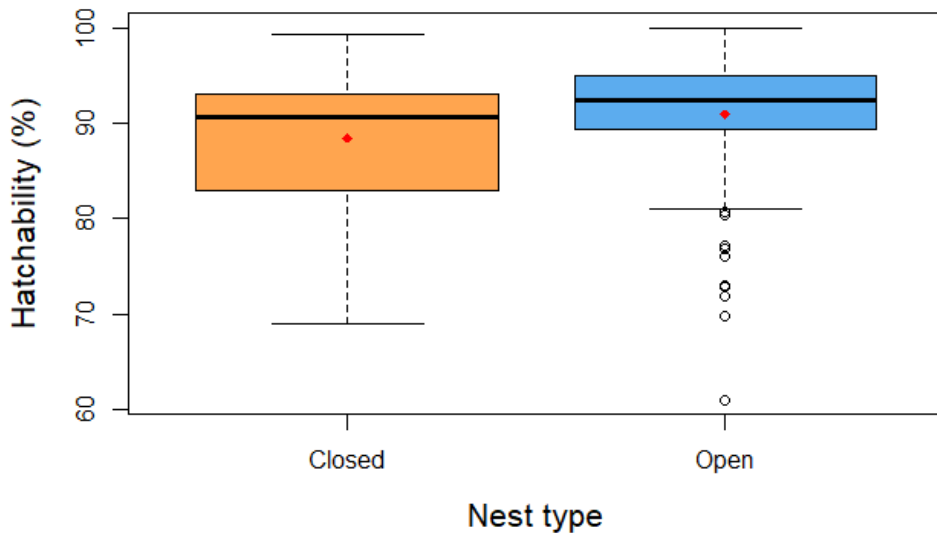


Figure 4: A boxplot showing the difference in hatchability between birds having open ($n=106$) and closed ($n=63$) nests. Red dots are mean values. Closed nests have a mean hatchability of 88.44% whereas the mean for open nests is 90.92%, this being a significant difference.

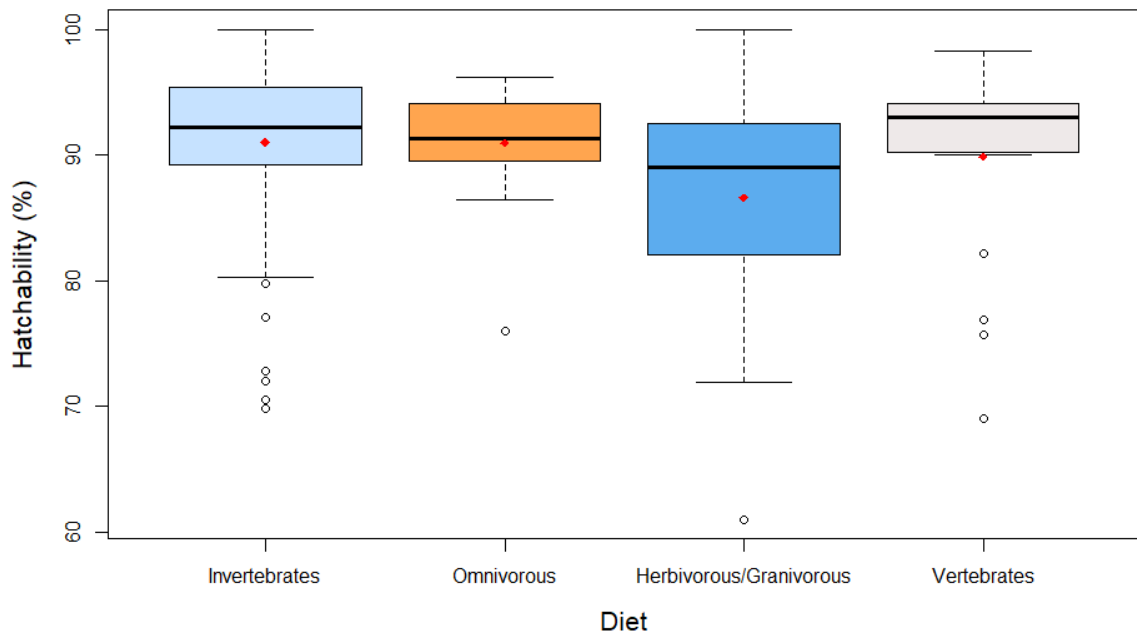


Figure 5: A boxplot representing hatchability for birds with different diets. The red dots represent the mean hatchabilities of the groups and are 91.01% for the birds eating invertebrates ($n=100$), 90.96% in omnivores ($n=16$), 89.97% in vertebrate-eating birds ($n=19$) and 86.62% in herbivores/granivores ($n=34$). The mean hatchability of herbivorous/granivorous birds is significantly different from the means of the omnivores and the birds that eat invertebrates.

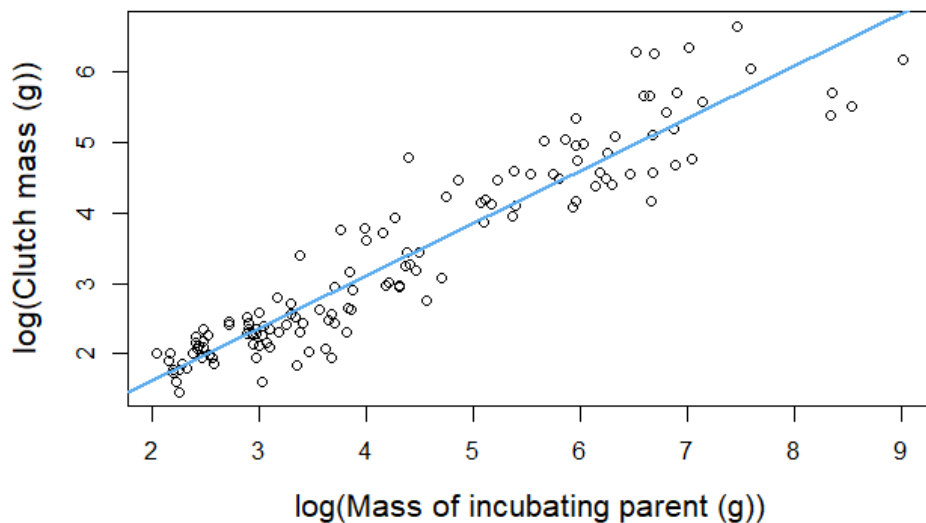


Figure 6: A regression illustrating the relationship between clutch mass and the weight of the incubating bird. Clutch mass increases together with the mass of the incubator. The linear expression of the regression line is $y = 0.56x + 1.49$.

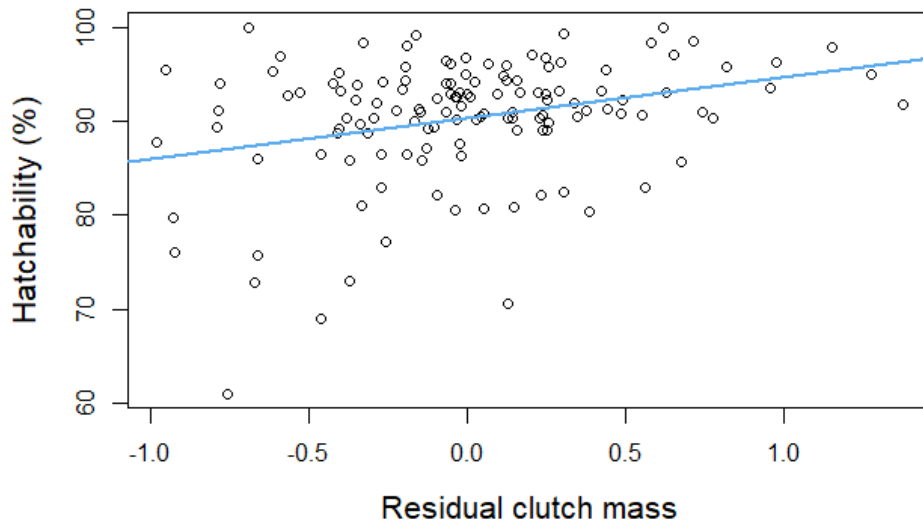


Figure 7: The regression shows that hatchability increases when relative clutch mass increase. The clutch masses that are relatively large compared to the body mass of the incubator also have high hatchabilities. The linear expression of the regression line is $y = 4.35x + 90.4$

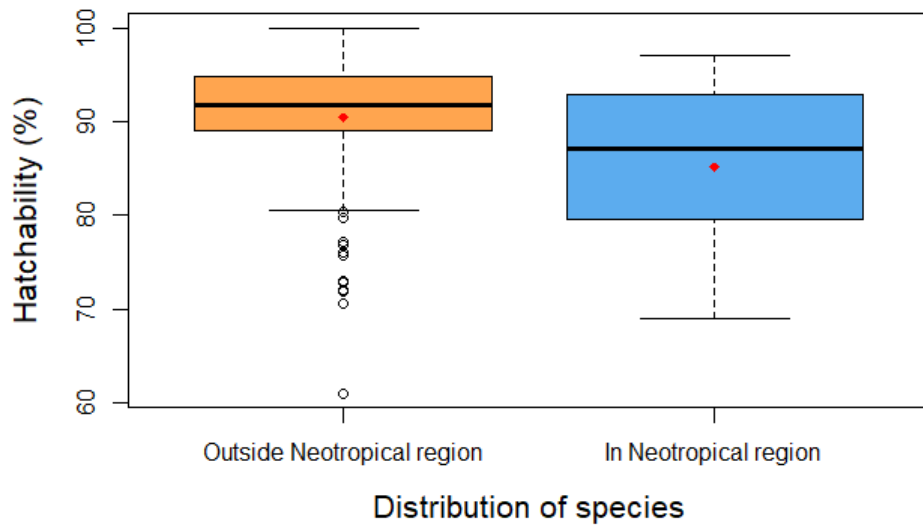


Figure 8: Species in the Neotropical fauna-geographic region have a lower hatchability than species that are distributed in the remaining parts of the world. The difference in mean hatchability (red dots) is 85.16% versus 90.47%.

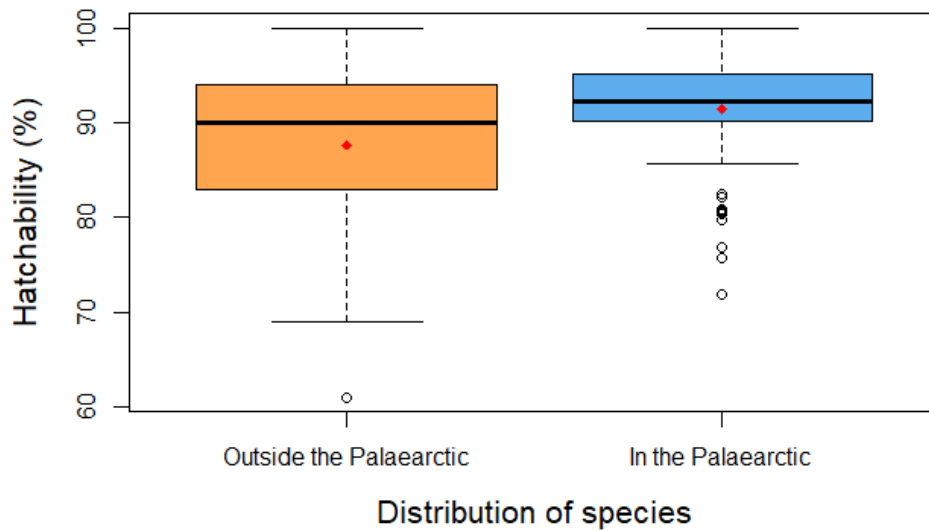


Figure 9: The boxplot shows bird species in and outside of the Palaearctic, with red dots representing mean hatchability. Species of the Palaearctic region have a mean hatchability of 91.23%, a significant higher value compared to 87.54% in the species that are not a part of this region.

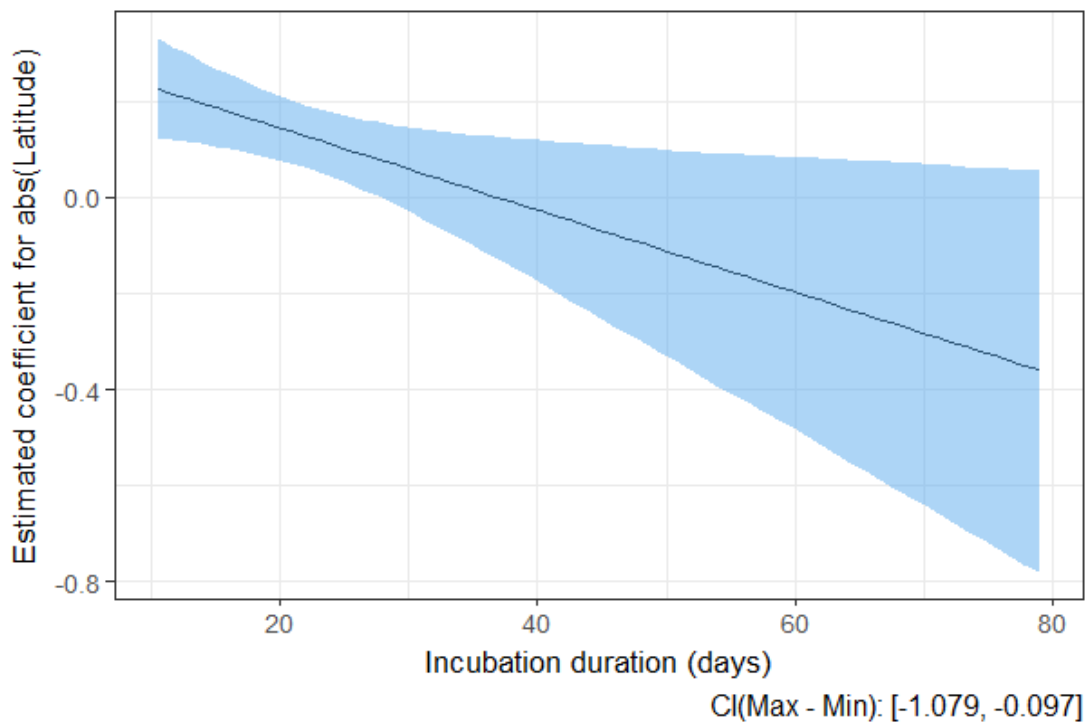


Figure 10: This plot shows the interaction between the continuous variables “absolute latitude” and “incubation duration” on hatchability. As the duration of incubation increases, the effect of abs(latitude) on hatchability decreases. The blue area is the 95% confidence interval. The maximal deviation from the line is -1.079, and the minimal deviation is -0.097.

Tables

Table 1: Overview of correlation between the continuous variables using Pearson's correlation coefficient. The correlation coefficients are above the diagonal, and the sample size n is below. A coefficient higher than 0.6 or lower than -0.6 is considered to be correlated and is emphasized in bold. The asterisk symbolizes the significance of the P-value, *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$.

	Latitude	Mass of incubating parent	Total clutch mass	Incubation duration	Residual clutch mass
Latitude	-	0.208*	0.153	0.165*	0.484***
Mass of incubating parent	315	-	0.602***	0.667***	-0.214
Clutch mass	315	292	-	0.496***	0.370***
Incubation duration	338	315	315	-	-0.061
Residual clutch mass	300	277	277	300	-

Table 2: Predictors excluded before model selection because of correlation or missing data.

Predictors	Explanation
Mass of incubating parent	Due to correlation with the variable "incubation duration"
Social organization	Due to missing data for 42 species
Incubator	Due to missing data for 20 species
Clutch mass	Due to missing data for 23 species
Residual clutch mass	Due to missing data for 38 species

Table 3: Descriptives of the continuous predictors, including minimum value, maximum value and median. abs(Latitude) is the absolute value of latitude, making all values positive and thereby converting the variable to the latitudinal distance from equator.

Predictor	Minimum value	Maximum value	Median
Incubation duration (days)	10.50	79.00	16.60
Clutch size (number of eggs)	1	15	4
Egg mass (g)	0.95	484.00	6.50
Clutch mass (g)	4.32	775.56	19.96
Mass of incubating parent (g)	7.80	8190.00	49.35
Latitude (decimal degrees)	-77.22	78.22	47.12
abs(Latitude) (decimal degrees)	0	78.22	48.00

Table 4a: Results from univariate PGLS-analyses of continuous predictors against hatchability. λ represents the degree of phylogenetic dependence in the relationship, spanning from 0 (no dependence) to 1 (full dependence). Adjusted R^2 is the proportion of total variance explained by the model, adjusted for the number of predictors included. DF is degrees of freedom. The asterisk symbolizes the significance of the P-value, *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$.

Predictor	N	Estimate \pm SE	T-value	P-value	λ	Adjusted R^2	DF
Mass of incubating parent	146	$-9.68 \times 10^{-4} \pm 6.52 \times 10^{-4}$	-1.49	0.14	0.67	8.26×10^{-3}	144
Clutch size	157	0.42 ± 0.25	1.71	0.09	0	0.01	155
Clutch mass	146	$6.62 \times 10^{-5} \pm 4.98 \times 10^{-5}$	1.33	0.19	0	5.26×10^{-3}	144
Incubation duration	169	-0.06 ± 0.07	-0.88	0.38	0.63	-1.30×10^{-3}	167
Absolute latitude	169	0.12 ± 0.03	3.61	4.07×10^{-4} ***	0.52	0.07	167
Residual clutch mass	131	4.35 ± 1.21	3.60	4.52×10^{-4} ***	0	0.08	129

Table 4b: Results from univariate PGLS-analyses of categorical predictors against hatchability. λ represents the degree of phylogenetic dependence in the relationship, spanning from 0 (no dependence) to 1 (full dependence). Adjusted R^2 is the proportion of total variance explained by the model, adjusted for the number of predictors included. DF is degrees of freedom. The asterisk symbolizes the significance of the P-value, *=P<0.05, **=P<0.01, ***=P<0.001.

Predictor	N	Mean squares	F-value	P-value	λ	Adjusted R^2	DF
Diet	169	2.42×10^{-8}	3.58	0.02 *	0	0.04	165
Incubator	149	2.56×10^{-10}	0.04	0.85	0,71	-6.55×10^{-3}	147
Social organization	127	1.10×10^{-4}	2.09	0.10	0	0.03	123
Nest type	169	2.44×10^{-4}	5.00	0.03 *	0	0.02	167

Table 5: The results from the minimal adequate model.

abs(Latitude) is the absolute value of latitude, making all values positive and thereby converting the variable to the latitudinal distance from equator. The asterisk symbolizes the significance of the P-value, *=P<0.05, **=P<0.01, ***=P<0.001.

Minimal adequate model: P-value= 12.1×10^{-4} ***, Adjusted $R^2=0.12$, $\lambda=0$.				
Predictor	DF	Mean squares	F-value	P-value
abs(Latitude)	1	6.41×10^{-4}	14.62	1.87×10^{-4} ***
Diet	3	1.26×10^{-4}	2.86	0.04 *
Incubation duration	1	5.61×10^{-5}	1.28	0.26
abs(Latitude):Incubation duration	1	2.19×10^{-4}	5.00	0.03 *