Why and how brain size evolves
Sociality, predation and allometry

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Academic dissertation for the Degree of Doctor of Philosophy in Ethology at Stockholm University to be publicly defended on Friday 9 February 2018 at 13.00 in Vivi Täckholmsalen (Q-salen), NPQ-huset, Svante Arrhenius väg 20.

Abstract

The evolution of the vertebrate brain has remained a topic of intense interest from biologists over many decades. Evolutionary biologists have seen it as an intriguing example of how the size and structure of a trait evolves across large phylogenies and under body size constraints, with both large shifts in deep evolutionary time and continuous smaller scale adaptation. Behavioral ecologists, on the other hand, have put great effort in trying to understand the costs and benefits of brain size and structural variation, usually assuming that the brain morphology of species is the result of a balance between energetic costs and cognitive benefits.

I discuss two hypotheses that aim to explain under what circumstances a higher cognitive ability yields fitness benefits. The predation avoidance hypothesis states that large brains help to avoid predators. The social brain hypothesis predicts that cognition is especially beneficial for animals living in complex social environments. In practice these hypotheses are difficult to differentiate (paper I), as sociality often evolves in response to predation pressure. Comparative studies on either hypothesis should therefore aim to control for effects of the other hypothesis, and experiments may be especially useful in testing more explicit mechanistic explanations.

I put the predation hypothesis to the test using two approaches, a comparative analysis and a within-species experiment. The comparative analysis (paper II) used published data on hawk predation and related it to both relative brain size and relative telencephalon size. While sparrowhawk predation was unrelated to brain morphology, birds that experience more goshawk predation had larger brains and telencephali. Next, I performed an experiment (paper III) on guppies that had been artificially selected for relative brain size. The selection lines have demonstrated differences in cognitive ability, as well as a marked survival difference under predation in females. I exposed fish to either a predator model or a novel object control, varying both sex and group size. Large-brained females performed fewer and shorter predator inspections than small-brained females, while keeping a larger distance from the predator model.

I performed another experiment (paper IV) to investigate differences in social competence. I calculated the duration of contests between random pairs of small- and large-brained males, using movement data. When the loser was large-brained, contests were decided almost 40 minutes earlier than when the loser was small-brained, indicating that the decision for the loser to give up is made quicker with a larger brain.

This thesis ends with an exploration of variation in the scaling relationship between brain and body size across vertebrates (paper V). The observed scaling between brain and body depends on what taxonomic level is under investigation. This effect, however, exclusively occurs in the two classes with the largest brains, mammals and birds. This indicates that strong developmental constraints have been alleviated in the two highly encephalized classes, but not elsewhere.

In conclusion, I find evidence that both predator avoidance and social factors may contribute to the evolution of brain size. Further work on explicit behavioral frameworks for cognitive benefit hypotheses is likely to yield significant insight. Constraints in brain size may be hard to overcome and play an especially large role at a larger taxonomic scale.

Keywords: brain size, brain size evolution, encephalization, predation, predator inspection, sociality, animal contest, constraint.

Stockholm 2018
http://urn.kb.se/resolve?urn=urn:nbn:se:su:diva-150336

ISBN 978-91-7797-103-0
ISBN 978-91-7797-104-7

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WHY AND HOW BRAIN SIZE EVOLVES
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Wouter van der Bijl
"Gee, Brain. What are we going to do tonight?"

"The same thing we do every night, Pinky. Try to take over the world."

(Pinky and the Brain)
The thesis is based on the following articles, which are referred to in the text by their Roman numerals:


II  Van der Bijl, W., Tsuboi, M., Iwaniuk, A.N. & Kolm, N. Prey-predator interactions and the evolution of bird brain morphology. *Submitted manuscript.*


IV  Van der Bijl, W., Kotschal, A., Buechel, S.D. & Kolm, N. Cognitive ability and antagonistic social competence: contest duration depends on loser’s brain size. *Manuscript.*


**Candidate contributions to thesis articles**

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* Contribution Explanation

Minor: contributed in some way, but contribution was limited.

Significant: provided a significant contribution to the work.

Substantial: took the lead role and performed the majority of the work.
I am also a (co-)author in the following articles that were written during my doctoral studies, but are not included in this thesis:


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Introduction

Evolving cognitive ability

Scientists over many generations, and across many academic fields, have been fascinated by cognition as a topic of study. Some perhaps because cognition is fundamental to the experience of the world and ourselves. Others perhaps because of the role that cognition has played in the evolution of animal life, tightly linked to behavior. In the case of evolutionary biology, the main objective is typically to explain variation in cognitive ability among animals, including humans (Shettleworth, 1998). More often than not, this is biased towards explaining increases in cognitive ability rather than the reverse. This can be understood to reflect a fascination with the complex, advanced, or “more human”, or simply that the ancestral state of cognitive ability in animals was likely low. So, broadly speaking, evolutionary studies of cognition typically aim to explain why some animals have evolved high cognitive ability, while others have done so to a lesser degree.

However, cognitive ability proves to be difficult to study. Let us use the common and useful definition of cognition by Sara Shettleworth (1998): “cognition, broadly defined, includes perception, learning, memory and decision making, in short all ways in which animals take in information about the world through the senses, process, retain and decide to act on it.” Cognitive ability is then the ability of animals to perceive, learn, remember and make decisions. These abilities can be observed, albeit only indirectly, by the study of behavior. Like school testing, if we give two animals a cognitive task, such as an associative learning task, the animal that consistently performs better can be assigned a higher cognitive ability. These sorts of approaches have been mainly used to study the presence or absence of “advanced” cognitive functions in highly cognitive species, although recently some broad and ambitious studies have performed such tests on sets of lab and zoo animals (Benson-Amram, Dantzer, Stricker, Swanson, & Holekamp, 2016; MacLean et al., 2014). However,
testing many individuals is logistically challenging especially if one wants to test for multiple cognitive domains. In addition, extending this approach to the between species level is often problematic, since differences among species in ecology, neophobia and morphology can affect performance.

**Brain size and cognitive ability**

Instead of inferring cognitive ability from behavior, one can also use the morphology of the substrate of cognition, the brain. Indeed, the study of brain size as an indicator of cognitive ability has a long history. For example, Dujardin (1850) measured brain size in bees, and Francis Galton (1889) famously related the head size of Cambridge students to their academic performance. The reasoning is straightforward, if cognition is limited by computational power, and larger brains have more computational units, cognitive ability is likely higher in larger brained animals. Brain size has been used in many studies on cognitive ability, especially in evolutionary studies (e.g. Byrne & Whiten, 1988; Clutton-Brock & Harvey, 1980; Dujardin, 1850).

Nonetheless, brain size has received considerable criticism. After Galton got associated with the eugenics movement and the atrocities of the Second World War, strong resistance against its use in humans became established opinion. The most public was perhaps Stephen Jay Gould’s book “*The mismeasure of man*” (1981), which attacked both craniology and IQ measurements. Brain size is indeed clearly a rough and simplistic measure of cognitive ability. It ignores the regional differences and detailed brain structure, which underlies specific functions (Striedter, 2006), although region sizes can be used in analyses when available. It also ignores the different scaling rules of neurons and fibers with brain size in different taxa, which may be better predictors than brain size itself, and these can certainly vary substantially (Herculano-Houzel, 2017). For example, the density of neurons in some bird brains is very high (Olkowicz et al., 2016), which means that using brain size to predict the cognitive ability of birds would likely give an
estimate that is too low. Neuronal scaling rules appear reasonably well preserved within many orders, though, so brain size comparisons at a smaller phylogenetic scale are likely to be relatively unaffected. A third critique is that not only an increase in computational units can increase intelligence, but also changes in wiring and connections, sometimes called algorithmic evolution (Chittka, Rossiter, Skorupski, & Fernando, 2012; Lihoreau, Latty, & Chittka, 2012).

Despite these critiques and theoretical arguments why the explanatory power of brain size may be limited, actual evidence is strong in support of brain size being a good proxy for cognitive ability. After long-lasting debate the consensus is now that brain size and IQ in humans are correlated, and the estimate from a meta-analysis on in vivo measurements estimates the population correlation at 0.33 in humans (McDaniel, 2005). In primate species, brain size related to their average performance in a set of nine cognitive paradigms (Deaner, Isler, Burkart, & van Schaik, 2007). Brain size predicted the cognitive ability of self-control (e.g. inhibiting a direct response to a food item) in 36 mammal and bird species (MacLean et al., 2014), as well as the ability to solve a puzzle box in 39 carnivore species (Benson-Amram et al., 2016).

In addition, brain size also appears to be important for cognitive ability within species. Strong evidence comes from experiments by Kolm, Kotrschal and colleagues, whom selected for small and large relative brain size in guppies (Poecilia reticulata) (Kotrschal et al., 2013). Based on a large number of assays, there are significant differences in the cognitive performance of these selection lines. Large-brained females outperformed their small-brained counterparts in both a numerical learning task (Kotrschal et al., 2013) as well as the reversal of a learned color association (Buechel, Bousard, Kotrschal, van der Bijl, & Kolm, n.d.), while large-brain males were quicker to learn the route through a maze than small-brained males (Kotrschal, Corral-Lopez, Amcoff, & Kolm, 2015). Considering these studies, while brain size certainly has limitations, it can explain substantial
variation in cognitive performance within and between species nonetheless, which could indicate that the influence of those limitations may be limited to comparisons on a large phylogenetic scope or certain taxa.

**Evolving brain size**

To understand the evolution of cognition then, one can make significant steps by studying the evolution of brain size. This fits comfortably in a paradigm of trade-offs, where brains are expected to be the optimal size when costs and benefits are balanced (Jerison, 1973). As will become clear towards the end of this thesis, this does not hold in practice, since evolutionary constraints can play a large role. But it is nonetheless a useful framework in which to consider brain evolution. The cost of brain size is usually seen as energetic, since the brain is expensive to produce and maintain (Aiello & Wheeler, 1995). These energetic costs can be visible in trade-offs, for example with other expensive organs such as the gut (Aiello & Wheeler, 1995; Kotrschal et al., 2013; Tsuboi et al., 2015) or testes (Pitnick, Jones, & Wilkinson, 2006), by life-history trade-offs such as lower fecundity or a longer development time (Gonzalez-Voyer, González-Suárez, Vilà, & Revilla, 2016; Kotrschal, Corral-Lopez, Szidat, & Kolm, 2015) or by decreased immune system function (Kotrschal, Kolm, Penn, & Kotrschal, 2016). A more extensive discussion of the costs of brain size is beyond the scope of this thesis, but it is important to recognize their role in limiting brain expansion, and that these costs may also vary across species.

Large brains are beneficial by providing cognitive advantages. While we have discussed some links between brain size and cognitive ability above, that data is about relatively abstract cognitive tests and therefore quite far removed from a selective advantage. One can show that self-control is better in large brained animals, but that does not immediately help us in understanding why certain species have larger brains than others unless we know what species need self-control for. A guppy never needs to count symbols on a wall, so the counting of
symbols is never selected for directly. However, the basic cognitive ability of numerosity may be beneficial in an ecological setting, such as when choosing between two social groups of different sizes to maximize anti-predator benefits. It is that step that is always suggested, but data directly supporting such links is often missing. To fill in the gap from the capabilities of large brains to the evolution of large brains, we need ecological theories that explain how the evolutionary benefit of cognitive ability arises, and explicitly test their predictions. That is, under which conditions do we expect that cognitive ability is selected for, and why? Many such hypotheses have been proposed, and they are typically evaluated by relating variation in ecological variables (in a broad sense, including social variables) to variation in brain size. This thesis focuses on two such hypotheses. First the predation avoidance hypothesis, which has received a lot of interest in the last few years, and second the social brain hypothesis, a more established and dominant hypothesis in brain size evolution. I summarize the key points here, but a longer discussion of both hypotheses and their connections is found in paper I.

The predation avoidance hypothesis

Predation is a very wide-spread and strong selection force (Dawkins & Krebs, 1979), that has strongly driven the evolution of many traits and innovations, such as coloration (Endler, 1978), nocturnality (Rydell & Speakman, 1995) and social grouping (Clutton-Brock & Janson, 2012; Groenewoud et al., 2016). This also includes specialized behaviors such as the detection, avoidance and escape to predators (Lima & Dill, 1990). On top of that, the expression of many other behaviors like foraging and mating related behaviors are modulated by the presence of predators (Caro, 2005). As all these behaviors are produced and modulated by the brain, there is a wide scope for predation to be important in the evolution of brain size. If larger brains would allow animals to better detect, avoid or escape predators this can yield strong positive selection on brain size (Jerison, 1973).
Jerison (1973) described this idea after he found that ungulates in the fossil record had larger brains when sharing a continent with large carnivores, compared to ungulates that were not under such predation pressure. In birds, brain size is related to their flight initiation distance which has been proposed to reflect their ability to monitor prey (Møller & Erritzoe, 2014; Samia, Møller, & Blumstein, 2015), although this result is not always replicated (Guay, Weston, Symonds, & Glover, 2013; Symonds, Weston, Robinson, & Guay, 2014). Good evidence comes from common eiders, where large-brained females lose fewer broods to predators under heavy predation (Öst & Jaatinen, 2015). Mammals that have physical predator protection, such as armor or quails, have smaller brains, possibly because they do not rely as much on behavioral strategies to deal with predation (Stankowich & Romero, 2017). Perhaps the strongest evidence comes from the brain selected guppy lines, discussed earlier, where a large experiment in semi-natural streams revealed that large-brained females survive significantly better under predation threat over the course of five months (Kotrschal, Buechel, et al., 2015).

The social brain hypothesis
A variety of separate but related hypotheses about the benefits of cognitive ability have been proposed that all center around challenges to individuals that are generated by group living. Early influences were the writings of Jolly (1966) and Humphrey (1976) that argued about the complexities of group living and the social environment. Humphrey likened social interactions to playing chess, where one needs reasoning to succeed and cannot rely on knowledge alone. Some resulting hypotheses are the Machiavellian intelligence hypothesis (Byrne & Whiten, 1988), where scheming and plotting of rivals in primate groups were central, and the cooperative breeding brain hypothesis (Burkart, Hrdy, & Van Schaik, 2009). But perhaps the most influential has been the social brain hypothesis (SBH) as posited by Robin Dunbar (1998) that also relates brain size to the complexities of group living. One might consider this a rebranding of the
Machiavellian intelligence hypothesis, as to be less focused on antagonistic interactions (Dunbar, 1998; Dunbar & Shultz, 2007). The core idea is that animals face ecological problems (in the narrow sense, excluding social factors), that they often solve with social mechanisms. It is then these social solutions that may require a larger brain (Dunbar & Shultz, 2017). While early on, group size was used as a proxy of “social complexity”, the focus was later placed on social bonds or pair bonds (Dunbar & Shultz, 2007; Shultz & Dunbar, 2010), although the main premise of SBH could arguably still hold with other measures of social complexity.

The evidence for the SBH is mixed. It has long relied on the correlation of group size and brain size in primates. This relationship was until very recently called “remarkably robust” and one of six “key empirical findings” that any theory of primate brain size needed to explain (Dunbar & Shultz, 2017). However, recent analyses on larger datasets now show that this relationship likely does not hold (DeCasien, Williams, & Higham, 2017; Powell, Isler, & Barton, 2017). In addition, the extension of the social brain hypothesis to other vertebrate species has not been very successful, although there may be effects of social structure at least in ungulates (Pérez-Barbería & Gordon, 2005; Pérez-Barbería, Shultz, & Dunbar, 2007) and birds (Shultz & Dunbar, 2010).

**Shared reasoning and challenges**

Although the predation avoidance hypothesis and social brain hypothesis are separate hypotheses with separate predictions, some ideas surface in both. Both hypotheses deal with biotic interactions, with the difference being whether these are inter- or intraspecific. But a lot of the intuitive background, such as the comparison with chess reasoning by Humphrey, are equally applicable to both. It is likely that biotic interactions combined explain substantial variation in brain size across vertebrates.
An issue for comparative analyses testing either hypothesis, is that predation pressure and sociality are likely to be correlated. Predation is the main, but not only, driver of the evolution of sociality in many systems (Isbell, 1994; Krause & Ruxton, 2002; Magurran & Seghers, 1994; Seghers, 1974; Shultz, Opie, & Atkinson, 2011). Therefore, analyses that consider only predation or only sociality, may conflate effects of both. Comparative analyses can only differentiate between predation and sociality if both factors are included, which they almost never are. **Paper II** tests for both these hypotheses simultaneously in birds.

A second issue for both hypotheses, is that the cognitive arguments are often verbal and somewhat vague. In the end, predation and sociality do not directly select for brain size, selection is mediated by the competence with which certain behaviors are performed. What these behaviors exactly are is often unclear and rarely tested. **Papers III and IV** investigate the relationship between brain size and behavioral mechanisms of both the predation avoidance and social brain hypothesis respectively.

### The studies and their methods

#### Comparative analyses

Both **paper II** and **V** use phylogenetic comparative methods, which are designed to take into account the shared ancestry between species (Felsenstein, 1985). In **paper II** I collated data from the literature on 94 bird species to test whether brain morphology was related to predation and sociality. Specifically, I included data on brain size, body size, telencephalon size, normalized predation rate, several social variables, diet and life history. Using these variables, I then tested for effects of sociality and predation on body size, relative brain size and telencephalon size, while controlling for diet and life history traits.

Since there were two separate predator species, the goshawk and sparrowhawk, both for which the data was incomplete, I ran separate analyses for the predators
to maximize sample size. For each analysis, I performed model selection on a consensus phylogeny using phylogenetic generalized least squares (PGLS). Then, to also consider the uncertainty in the phylogeny, I fitted the selected models on 500 phylogenies using a Gibbs sampler (Hadfield, 2010) and merged the chains afterwards (Guillerme & Healy, 2014; Healy et al., 2014). Finally, as previous authors had proposed that the link between brain size and predation is mediated by flight initiation distance, I tested that hypothesis directly using phylogenetic path analysis (van der Bijl, 2017; von Hardenberg & Gonzalez-Voyer, 2013).

**Paper V** uses both published and unpublished data on brain and body sizes, with more than 20,000 specimens for over 4,500 species, across vertebrates (Figure 1). This wealth of data was then used to describe the allometry between brain and body size in detail. I investigated the taxon-level effect (TLE, Lande, 1979), that describes how the allometric slopes varies by what taxonomic level is used. Generally, allometric slopes are believed to be steeper when calculated at higher taxonomic levels, and shallowest when comparing individuals within a species. I investigated this within the six vertebrate classes by fitting PGLS models at the class, order, family and genus levels as well as linear models at the species levels. In addition, I also fitted PGLS models at each internal node of the phylogeny, to investigate the TLE on a continuous time scale. Ornstein-Uhlenbeck (OU) models were used to estimate the conditional variance and rate of evolution of relative brain size within clades, to further elucidate differences between small- and large-brained vertebrates.
Figure 1 | Brain-body allometry of six major vertebrate classes. a, Class level brain-body allometry is estimated by phylogenetic generalized least squares (PGLS). b, Minimum convex polygons of six major vertebrate classes highlight that Mammalia (N = 1409) and Aves (N = 1902) occupy morphospace above Actinopterygii (N = 963), Amphibia (N = 86), and 'Reptilia' (N = 79), while Chondrichthyes (N = 147) significantly overlap with both levels of encephalization.

**Behavioral tests of guppies**

In addition to the macro-evolutionary analyses I also performed experiments on guppies (*Poecilia reticulata*) in papers III and IV. Both these experiments use guppies that had been artificially selected for small and large relative brain size (Kotrschal et al., 2013). In short, random pairs of guppies were allowed to reproduce, and when they had produced sufficient offspring they were sacrificed, and their brains were weighed. Parents with the largest and smallest 20% combined brain size were then used to establish an up- and down-selected line. This procedure was performed three times to create three replicates. After three
generations of selection a difference of more than 10% had been formed between the lines.

As discussed earlier, the small- and large brain guppies differ in their cognitive ability (Buechel et al., n.d.; Kotrschal et al., 2013; Kotrschal, Corral-Lopez, Amcoff, et al., 2015). Micro-CT imaging has shown that all brain regions have responded to the selection and have increased according to normal brain allometry (Kotrschal et al., 2017). Interestingly, large-brained females survived better under predation that small-brained females (Kotrschal, Buechel, et al., 2015). Paper III investigated the behavioral mechanisms behind this difference.

For this, I exposed a total of 336 fish in 144 groups to both a predator model and a novel object control (Figure 2). After acclimating in an experimental tank overnight, shoals of 1, 2 or 4 fish, separated by brain size and sex, were given both stimuli in random order and with several hours in between, and were recorded for 20 minutes. These videos were then analyzed in detail afterwards. First, all discrete behaviors were scored manually using the JWatcher software (Blumstein, Daniel, & Evans, n.d.). Guppies perform so-called predator inspections towards predators where they approach the predator, often with an angled posture (Dugatkin & Godin, 1992). The number of these inspections, their total duration and their mean duration were extracted from the videos by an observed who was blinded to the brain size of the groups.

In addition, to form a better idea about the actual risk the fish would be in, I used computer vision software Ctrax (Branson, Robie, Bender, Perona, & Dickinson, 2009) to track the location of the fish during the trials. I then made grid heatmaps from this positional data describing which areas of the tank, relative to the predator model or novel object control, were visited more or less than average. I also compared the observation density between small- and large-brained fish at the different tank positions.
Figure 2: The stimuli used in paper III, with two painted fishing lures as predator models, and a coffee cup as a novel object control

In paper IV I investigated the contest behavior of guppy males. We had previously hypothesized (paper I) that large-brained individuals are better at assessing their performance in a contest. This leads to the prediction that dominance should be established faster, if the subordinate male has a large brain. To this end, I needed to establish a method to quantify contest duration in guppy males.

For this, in a first experiment I made 16 randomly matched pairs of wild-type males. These males were put on either end of a tank with a removable wall separating them, and left to acclimate overnight. The following morning the wall was removed and the fish were free to interact for two hours. Again, I recorded the interaction with an overhead camera, and annotated the videos with both manual counts of discrete behavior and tracking with computer vision software. I counted all attacks between the males. In addition, I quantified the displacement between the two males in continuous time by calculating a simple displacement statistic (Figure 3).

In a second experiment I used small- and large-brained males, from the selection lines discussed previously, and hosted contests between them according to the expectation of random encounters. In this full-factorial design there were 16 small vs. small, 16 large vs. large and 36 small- versus large-brained contests. The setup
was identical to the first experiment, except that I recorded for three hours. After the contests, I quantified the size, weight and amount of coloration for all males.

To quantify contest duration, I estimated for each trial the moment when the fish had begun moving asymmetrically. That is, at the start of a contest, both fish are displacing each other. But after some time, when the dominance relationship is resolved, this displacement becomes asymmetrical, where the dominant individual displaces the subordinate more often. For each moment in a trial, I summarized the evidence by calculating a Bayesian t-test over the observations.

![Figure 3](image.png)

**Figure 3:** An example of a displacement event and the displacement metric during the event. a) Video snapshots during a displacement event, with red numbers indicating time in seconds. Overlaid are movement tracks used to perform the calculations. b) A timeline of 7 seconds for each of the variables and the calculated metric during the displacement event from panel a. Note that the light and dark blue lines match the light and dark blue tracks in panel a. The dark blue individual displaces the light blue individual, which is expressed in deviating displacement scores. The attraction reaches a peak at around 4 seconds. c) Illustrations of the three variables used to calculate displacement, speed, distance and social angle, and the formula to calculate the score.
up to that point. Once the evidence for asymmetry was strong enough (a Bayes factor of $10^{1/2}$, or around 3 times as likely as the alternative) I took that as the end point of the contest.

Using a model averaging approach, I investigated the contribution of the different variables to contest duration, including brain size of the winner and loser, as well as physical traits such as size, condition and coloration. The physical traits were used as both winner and loser trait values and their difference, as we do not know how guppies assess these traits.

Results and discussion

Paper II

Both the relative brain size estimate ([95%CI], 0.041 [0.002, 0.079], pMCMC = 0.043) and telencephalon size (0.150 [0.037, 0.257], pMCMC = 0.012) of the birds included in the study co-varied with the predation rate by goshawks. But no effects of sparrowhawk predation were apparent (brain size: -0.008 [-0.043, 0.027], pMCMC = 0.64; telencephalon size: 0.088 [-0.076, 0.265], pMCMC = 0.274). Surprisingly, I did not find any relationship between body size and predation, but this does make interpretation of the other effects simpler, as they are not confounded by body size.

In addition, I found that relative brain size was larger for sedentary and altricial birds, both previously well-known effects. Brains were also larger for birds that eat other vertebrates, as opposed to those eating invertebrates, plant material or a combination of those two. None of the social variables influenced brain morphology. There was no evidence supporting that the link between goshawk predation and brain morphology was mediated by flight initiation distance.

These results support the predator avoidance hypothesis, where bird species that are under stronger goshawk predation pressure may have evolved larger brains. In particular, the telencephali of highly predated species are larger than expected,
indicating that perhaps attention or learning processes are particularly selected for. It is unclear why there are effects of goshawk predation and not sparrowhawk predation, but one possible explanation is that the dataset for sparrowhawks contains less variation in size and is of a narrower phylogenetic scope, reducing our power. It remains an open question what behavioral mechanisms may underlie these results.

**Paper III**

The predator model was able to elicit a strong behavioral reaction, when compared to the novel object control. Like in the survival experiment, I found strong effects of brain size in females, but not in males. Specifically, large-brained females had much fewer and much shorter inspections than small-brained females (Figure 4). Using the tracking data, I confirmed that this put large-brained females further away from the predator, and therefore at lower risk (Figure 5). There is a clear ring shaped area around the predator that large-brained females visit much less often than small-brained females. Males were more cautious than females.

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**Figure 4**: Parameters of inspection behaviour for the different treatments presented as boxplots, indicating the median and quartiles with whiskers reaching up to 1.5 times the interquartile range. The violin plot outlines illustrate kernel probability density, i.e. the width of the shaded area represents the proportion of the data located there. Significance is based on LMMs (*p < 0.05, **p < 0.01 and ***p < 0.001). (a) Total time spent inspecting the predator model per fish in the shoal. (b) Total number of inspections per shoal. Note the use of log10 scales.
Figure 5: Overview of positional data during trials with a model predator, presented as statistical heat maps. First row (a) shows the overall result of each sex and the second row (b) splits this up per brain size. Each cell (pixel) in the heat maps shows the p-value of a non-parametric test. In the orange and blue maps, we tested against the median density to visualize the areas of the tanks that were visited more or less than expected. The pink and green maps show the statistical comparison between groups; i.e. males and females or small- and large-brained individuals. Black rectangles indicate predator position. Numbers between parentheses denote sample sizes. Note the nonlinear axis in the legend.

Overall, the brain size effects were specific to the predator model condition, and I found no differences in overall activity.

These results fit well with the survival difference between small- and large-brained females, and confirm that there is indeed a plausible behavioral mechanism behind the previously discovered difference in predation rate. It may be that the large-brained females need fewer visits to the predator model to assess it, or that they can assess it from a larger distance.

Paper IV
In the first male contest experiment, I found that measuring attacks is not a fruitful way to estimate contest duration. This is because the attacks occur
relatively late in the contest, and are already very one-sided at the time of the first attack. Therefore, dominance is likely to be established before the onset of attacks, and I could confirm this by showing that the displacement is already asymmetric five minutes before the first attack. This means that measuring displacement is necessary to determine contest duration in the guppy.

In the second experiment, contest duration was best predicted by the brain size of the loser. On average, contests where the loser was large-brained were on average 36 minutes shorter than those where the loser was small-brained (Figure 6, $SE_{\text{adjusted}} = 13.52$, $z = 2.66$, $p = 0.008$). The brain size of the winner, on the other hand, had no effect ($\beta = -18$, $SE_{\text{adjusted}} = 16.75$, $z = 1.072$, $p = 0.284$). Small- and large-brained males were equally likely to win the fight.

**Figure 6**: a) Estimated contest durations as a function of brain size of either the winner, or the loser. Boxplots show the raw data, while point estimates and error-bars indicate the predicted means and 95% confidence intervals by the average model, keeping all covariates at their mean value. b) Estimated effect of winner and loser brain size on contest duration with 95% confidence intervals. c) Variable importance of winner and loser brain size, as the proportion of top models (within 4 AICc of the best model) that each term is included in.
These results support the hypothesis that brain size may be important in antagonistic social competence. When males need to assess their own and/or their opponents performance in the contest, they are likely using primarily visual cues. The large-brained males are faster in using that information to decide to give up if they are losing.

**Paper V**

The taxon-level effect (TLE), long thought to be universal, is only present in the two highly encephalized classes, mammals and birds (Figure 7). In the other classes, the allometric slope is nearly constant regardless of whether it is at the within-species level or class level. This suggests that there are strong ontogenetic constraints, that have been weakened in mammals and birds, leading to a partial decoupling in brain-body allometry. The reduction in allometric constraint has coincided with the encephalization events.

However, there is no indication that there is more conditional variance of brain size in mammal and birds, that is, brain size is not “evolving more” in those classes. Instead, it is the within-species allometry that is highly variable in birds and mammals. This likely means that in these classes there is increased variance in brain ontogeny, while that has remained heavily constrained elsewhere. This ability to evolve these ontogenetic processes has likely allowed birds and mammals to evolve larger brains.
Conclusions and future directions

This thesis focuses mainly on two hypotheses of brain evolution, the predation avoidance hypothesis and the social brain hypothesis. These hypotheses aim to explain the selective advantage cognitive ability provides, are intimately linked in their underlying rationale, and are difficult to distinguish in comparative data. The work presented here aimed to contribute towards resolving these issues. First, I have provided a framework for effects of predation and sociality may be intertwined and highlighted the need for the inclusion of both hypotheses in comparative study, and the study of proximate behavioral mechanisms in the analyses of brain size (paper I). Second, I have presented a comparative analysis on bird brain morphology that includes data on both predation and sociality, allowing for a direct comparison between the two hypotheses, and found only predation to be of significant importance in my dataset (paper II).

Third, I have proposed reasonable behavioral mechanisms for both hypotheses, and given experimental evidence that they are related to brain size. The difference in risk taking and predator inspection can explain survival differences between
small- and large-brained females (paper III). The ideas behind the social brain hypothesis may apply to fish, although in a more classical antagonistic context (paper IV). The large differences in their assessment ability during a fight, and the ubiquity of animal contests, make these results potentially widely relevant. These results illustrate the powerful approach of integrative biology, where macro-evolutionary hypotheses are linked to proximate mechanisms. To continue to move forward with these explanations of brain size evolution, especially now that the comparative evidence has become more controversial, more behavioral approaches can prove to be important.

The results of the last paper (paper V) strongly emphasize the role of evolutionary constraints in the macro-evolution of brain size. It is not clear at all how much ecological hypotheses will have to say about the evolutionary patterns at very long time scales, such as the encephalization of birds and mammals. Perhaps the selection pressure for larger brains has been strongly present elsewhere, but the developmental constraints have never been resolved. Sharks and rays are an interesting group that will require further study, as their encephalization is intermediate between mammals and birds and the other classes. Nonetheless, brain size is clearly evolving at approximately equal levels in both encephalized and non-encephalized classes. Inter- and intraspecific interactions likely play a substantial role in driving the evolution of brain size across vertebrates.

References


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Svensk sammanfattning

Hjärnans evolution inom gruppen ryggradsdjur är ett ämne som har intresserat biologer under lång tid, och har studerats intensivt under flera decennier. Evolutionsbiologer betraktar det som ett fascinerande exempel på hur storlek och komposition av en biologisk egenskap utvecklas över evolutionär tid, begränsad av kroppsstorlek, med både stora förändringar över lång tid och småskalig anpassning över kort tid. Beteendeekologer, å sin sida, har lagt stor märka på att försöka förstå de nackdelar och fördelar som kommer med en viss storlek och komposition av hjärnan, vanligtvis med ett antagande om att den resulterande hjärnstorleken representerar en balans mellan energimässiga kostnader och kognitiva fördelar.


Jag testade ”predation avoidance”-hypotesen med hjälp av två metoder; en jämförande analys och ett inomartsexperiment. I den jämförande analysen (artikel II) användes tidigare publicerad data på predation från hökar, vilket jämfördes med relativ hjärnstorlek och relativ telencephalon-storlek hos bytestdjuren. Predation från sparvhök visade sig inte vara relaterat till hjärnmorfologi, men de fåglar som utsattes för högre predation från duvhök hade
både större hjärnor och större telencephali. Därefter utförde jag ett experiment (artikel III) på guppys som hade avlats på stor respektive liten relativ hjärnstorlek. Selektionslinjerna har tidigare uppvisat skillnader i kognitiv förmåga, och en tydlig skillnad i överlevnad hos honor under predation. Jag exponerade dessa guppyfiskar för antingen en predatormodell eller ett för dem okänt föremål (kontroll), där både kön och gruppstorlek varierades. Storhjärnade honor utförde färre och kortare så kallade ”predator inspections” än småhjärnade honor, och höll sig på ett större avstånd från predatormodellen.


Denna avhandling avslutas med en kartläggning av variationen i det allometriska förhållandet mellan hjärnstorlek och kroppsstorlek hos ryggradsdjur (artikel V). Det observerade förhållandet mellan hjärnstorlek och kroppsstorlek beror på vilken taxonomisk nivå som studeras. Denna effekt återfinns dock bara hos de två klasserna med de största hjärnorna, däggdjur och fåglar. Detta tyder på att starka utvecklingsmässiga begränsningar har lättats i de två klasser med störst relativ hjärnstorlek, men inte hos de andra klasserna.

Sammanfattningsvis har jag funnit stöd för att både predation och sociala faktorer bidrar till evolutionen av hjärnstorlek. Framtidiga studier som utarbetar tydligt definierade beteendemässiga ramverk över hypoteser som rör fördelarna med kognition kommer sannolikt bidra till större insikt framöver. Begränsningar på evolutionen av hjärnstorlek kan vara svåra att överkomma, och spelar därför en särskilt stor roll på högre taxonomiska nivåer.
Acknowledgments

I owe the largest thanks to my supervisor, Niclas, thank you so much for placing trust in me, and bringing me to Sweden to start this great adventure in Stockholm. From very early on, you allowed me to pick my own topics and define my own project. This has allowed to make and learn from my own mistakes (and many of them), but also to own the success when things did work out. I have always felt completely supported, whether it was with quick comments during writing, words of encouragement and suggestions after a failed experiment or supplying me with all the space and materials I needed. You always had my best interests in mind.

The growing research group you have fostered has been wonderful to work in, and the opportunities for collaboration (often encouraged by you) with many lab members have made my PhD both much more enjoyable and much more productive. Your ability to adjust and be an effective supervisor for a diverse set of people is unique, and something I can only aspire to. You were not just a great supervisor because you are a great scientist, but also because you are a great boss.

Alberto, I consider us PhD-brothers. As you had ample guppy experience and I had none, you had to take on the responsibilities of the big brother, and teach me how to work in a fish lab from the very basics. Any success I’ve had in the lab is also very much due to you. It has been wonderful to always have someone to bounce ideas off, and joining a (back then mostly Swedish) department as a pair was much easier. A second lab member with a very large influence over this thesis is Alex. You of course created the selection lines which two of these papers use. But also, you have played a big role since you have come Stockholm and often provided precious advice on my projects and alternate viewpoints to Niclas and a different style in general. Séverine, thank you for all support and help, and of course lots of fun collaboration. I have learned a lot from you in how you phrase
your research so meticulously, both in talks and in writing. Both you and Alex
have played a crucial role in making the Kolm lab what it is today.

I would like to thank my other collaborators, most notably Masahito and Andrew.
Thanks so much for involving me in these two wonderful projects. I have learned
so much about comparative analysis from you, and have a wider view of evolution
and brain size to thank for it. Also, a big thanks to Malin, after I caused some
start-up trouble for us I think we ended up with a nice paper that we can both be
proud of. Thank you, Henrik and Steph, for all your help and fun! Simon, thank
you so much for writing my Swedish summary, I’m sure it is great!

There are many people in the department whose advice and conversations have
been important in forming the ideas in this thesis. This obviously includes the
members of my follow-up committee, Olle, Gabriella and Kalle. Thank you for
helping me steer in a fruitful direction early on, and for providing feedback on
both this thesis and the licentiate. Björn, Alejandro, John, and Chris, your
influence is certainly felt within this thesis as well.

Without the wonderful behavioral biology group at Groningen university, I would
have never been here. Thank you, Ton, for inspiring me to study behavior in the
first place with your undergraduate lectures, and continued support and believe
in me. Thank you, Bernd and Simon, for your great supervision in my Master’s
projects. A great thanks as well to Jean-Christophe, Martine, Roelof, Domien,
Mirre, Jelle, Meghan and many others for letting me pester you with all my
questions, and making me feel welcome and appreciated. I cannot imagine a better
preparation to doing a PhD project than the education you all have given me.

I have worked in the department with great pleasure, certainly also because of the
great social atmosphere. Thanks to all of you that were common guests at the
Friday pub *Gyllene spiggen* and many other social events such as Halloween,
Christmas, whiskey club, etc. This includes Bertil, Ulf, the Wheats, Jason, Lovisa,
Christen, Simon, Meet, Olga, Timm, Philipp, Eva, Will, Jo, Ale, Charel, Ariel, Kyle, Sandra, Olle, Mariana, Raimondas, Sara, Ciaran, Yunfei and many others. A special thanks to Birgitta, who made me feel a very welcomed new student in the department. We have a great department, with so many friendly faces. I hope to return many times.

Thanks to all of those in the open software community, who have built the many tools that are used in this thesis free of charge, and without whom most of these papers could not exist. A special thanks to the crowd in the R-tag on StackOverflow, and the members of GMTs for helping me out and keeping me company.

I have had the fortune to grow up in a warm and supporting family. This adventure would have stopped a long time ago if my parents had not enabled my curiosity and encouraged me to pursue my interests. Thanks so much, Johan, Anita, Henriette and Jan. Also thanks to Bas and Jeroen, for pushing their youngest brother to be smarter and stronger. Thanks also to all my friends, back home and in Sweden, with special mentions for Kalahari, the B-track and hockey club Mesaicos. You have brought me so much fun throughout the years. And finally, a very special thanks to you, Amy. You unfortunately had to suffer the most from my stress and workload towards the end. I'm extremely grateful for all the help you have given, all the support and all the fun we continue to have together. Meeting you has been the best outcome of four years in Stockholm.