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Thinking in water

Brain size evolution in Cichlidae and Syngnathidae

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Abstract

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Brain size varies greatly among vertebrates. It has been proposed that the diversity of brain size is produced and maintained through a balance of adaptations to different types and levels of cognitive ability and constraints for adaptive evolution. Phylogenetic comparative studies have made major contributions to our understanding of brain size evolution. However, previous studies have nearly exclusively focused on mammalian and avian taxa and almost no attempts have been made to investigate brain size evolution in ectothermic vertebrates.

In my thesis, I studied brain size evolution in two groups of fish with extreme diversity in ecology, morphology and life history, Cichlidae and Syngnathidae. Using phylogenetic comparative methods, I investigated four key questions in vertebrate brain size evolution; cognitive adaptation, sexual selection, phenotypic integration and energetic constraints.

I have demonstrated i) that phenotypic integration can link functionally unrelated traits, and this may constrain independent evolution of each part involved or promote concerted evolution of an integrated whole, ii) that brain-body static allometry constrains the direction of brain size evolution, even though the static-allometry showed ability to evolve, allowing evolution of relative brain size under allometric constraints, iii) that the energetic constraints of development and maintenance of brain tissue is an important factor in forming the diversity in brain size in cichlids and syngnathids, both at macroevolutionary and microevolutionary time scales, and iv) that adaptation for feeding and female mating competition may have played key roles in the adaptive evolution of brain size in pipefishes and seahorses. To conclude, my thesis shows the strong benefit of using fish as a model system to study brain size evolution with a phylogenetic comparative framework.

Keywords: brain evolution, phylogenetic comparative method, the expensive tissue hypothesis, cichlid, pipefish, seahorse

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I   Tsuboi, M., Gonzalez-Voyer, A. and Kolm N. (2014) Phenotypic integration of brain size and head morphology in Lake Tanganyika cichlids. *BMC Evolutionary Biology* 14:39

II   Tsuboi, M., Kotrschal, A., Hayward, A., Buechel, D.S., Zidar, J., Løvlie, H. and Kolm N. Brain size evolution under allometric constraints in Lake Tanganyika cichlids. *Manuscript.*

III   Tsuboi, M., Husby, A., Kotrschal, A., Hayward, A., Buechel, D.S., Zidar, J., Løvlie, H. and Kolm N. (2014) Comparative support for the expensive tissue hypothesis: big brains are correlated with smaller gut and greater parental investment in Lake Tanganyika cichlids. *Evolution* 69:190-200

IV   Tsuboi, M., Shoji, J., Sogabe, A., Ahnesjö, I. and Kolm N. Within species support for the expensive tissue hypothesis: a negative association between brain size and visceral fat storage in females of Pacific seaweed pipefish. *Manuscript.*

V   Tsuboi, M., Lim, A.C.O., Ooi, B.L., Yip, M.Y., Chong, V.C., Ahnesjö, I. and Kolm N. Prey motility, egg size and female mating competition: Brain size evolution in pipefishes and seahorses. *Manuscript.*

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The following papers were written during the course of my doctoral studies but are not part of the present dissertation:

Tsuboi, M., Gonzalez-Voyer, A., Höglund, J. and Kolm N. (2011) Ecology and mating competition influence sexual size dimorphism in Tanganyikan cichlids. *Evolutionary Ecology* 26: 171-185

Tsuboi, M., Gonzalez-Voyer, A. and Kolm N. (2015) Functional coupling constrains craniofacial diversification in Lake Tanganyika cichlids. *Biology Letters* 11: 20141053

Hayward, A., Tsuboi, M., Owusu, C., Kotrschal, A., Buechel, D.S., Zidar, Charlie K. Cornwallis, J., Løvlie, H. and Kolm N. The influence of host ecology on parasite load: insights from Lake Tanganyika cichlids. *Submitted manuscript*.

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Abbreviations

ETH   Expensive tissue hypothesis
EBH   Expensive brain hypothesis
PGLS   Phylogenetic generalized least square
SnL/HL   Relative snout length compared to head length

Introduction

Vertebrate brain size evolution

The vertebrate brain is highly variable in size (Jerison 1973). Since Darwin (1871), evolutionary biologists have investigated the processes and mechanisms that produce and maintain the diversity of vertebrate brain size. The core concept that explains contemporary organic diversity is adaptation (Williams 1996; Futuyma 2013). Because variation in brain size underlies differences in cognitive performance (Kotrschal et al. 2013), brain size variation is considered to evolve in response to variation in the ecological and social environments under which different types and levels of cognitive capacity are favored (Striedter 2005). However, the trajectory and possibility of trait adaptation is constrained by various factors. The process of neural development (Lande 1979; Finlay and Darlington 1995; Striedter and Northcutt 2006) and the extensive energy budget required to develop and maintain neural tissue (Aiello and Wheeler 1995; Aiello et al. 2001) have been proposed to have profound influence on brain size diversification. The operation of these two general principles, i.e. adaptations and constraints, is further influenced by phylogenetic history, environment, behavior and the ontogeny of the animals in question. Therefore, the explanations of why and how vertebrate brains evolved to different sizes include a diverse set of interacting principles and factors (Striedter 2005).

Historically, the study of brain size in evolutionary biology stemmed from the observation that humans have considerably larger brains for a given body size compared to many, though not all, vertebrates (reviewed in Striedter 2005). It is therefore not surprising that subsequent attention in this research field focused on large brained animals, namely anthropoid primates and birds. However, these groups represent less than 20% of the overall vertebrates diversity. With approximately 26,000 species, teleost fish is by far the most species rich lineage of vertebrates (Helfman et al. 1997). The remarkable level of geographical, ecological and morphological diversification in fishes is matched by accompanying variation in their cognitive ability (Brown et al. 2008). The diversity of fishes therefore offers an exceptional opportunity to test various hypotheses on brain size evolution that were hitherto almost exclusively tested in mammalian and avian taxa.

Cognitive adaptation

There is ample evidence supporting that among-species variation in both absolute brain size and relative brain size (i.e. brain size after the effect of body size is removed) is shaped through adaptation to various cognitive challenges that animals face in their natural environment (Harvey and Krebs 1990; Striedter 2005). Ecological factors; diet (Cluttonbrock and Harvey 1980; Hutcheon et al. 2002), habitat complexity (Shultz and Dunbar 2006), predation (Kotrschal et al. 2015), social aspects; social group size (Barton et al. 1996; Dunbar and Shultz 2007), mating system (Barton 2006; Shultz and Dunbar 2006) and more directly cognition-related measures; tactical deception rate (Byrne and Corp 2004), social play (Iwaniuk et al. 2001; Pellis and Iwaniuk 2002; Graham 2011), innovation propensity (Lefebvre et al. 1997; Nicolakakis and Lefebvre 2000; Sol and Lefebvre 2000; Timmermans et al. 2000; Lefebvre et al. 2002), food hoarding (Garamszegi and Eens 2004; Garamszegi and Lucas 2005), success in a novel environment (Sol et al. 2005; Maklakov et al. 2011) are all have been proposed to affect and drive adaptive brain size evolution.

Phenotypic integration

Biological systems require their different parts to be integrated in order to function as a coherent whole (Gould and Lewontin 1979). The independent parts of the animal body may therefore represent evolutionary compromises due to the association in developmental, genetic, constructional and functional aspects of different traits (Olson and Miller 1958; Pigliucci and Preston 2004). In studies of brain size evolution, it has been a long standing observation that the size of the brain is explained to a large extent by variation in overall body size in form of a simple power law, $Y = aX^b$, where X is body size, Y is brain size (figure 1). Such a pattern of covariation is referred to as allometry (Huxley 1924; Huxley 1932). Various mechanisms have been suggested to produce patterns of brain size allometry, such as body surface area to brain volume scaling (Jerison 1973), metabolic constraints (Martin 1981) and a genetic correlation between body size and brain size (Lande 1979). In all of these mechanistic explanations, the underlying idea is that the tight covariation between brain and body size indicates that independent evolution of brain size is constrained by a constant growth regulation (Huxley 1932; Simpson 1944; Jerison 1973; Gould and Lewontin 1979; Lande 1979).
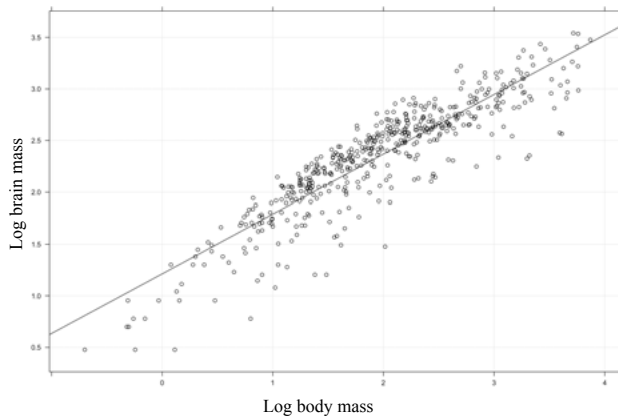
Figure 1. Relationship between brain mass and body mass for 489 marine teleost fish species in a double logarithmic scale. Slope (b) = 0.58, Intercept (a) = 1.21, R^2 = 0.81. Data taken from Iglesias *et al.* 2015.

Furthermore, the development of the brain is tightly integrated with development of the neurocranium (Richtsmeier et al. 2006; Richtsmeier and Flaherty 2013). As a consequence, diversification of brain size has coincided with evolution in cranial morphology. In mammals and birds, evolutionary increase in brain size (i.e. encephalization) has occurred through an accompanying increase in skull size (Iwaniuk and Nelson 2002; Luo 2007; Koyabu et al. 2014) and reduction in size of the feeding apparatus (Stedman et al. 2004). In fish, the telencephalon, one of the structural parts of the brain, develops through a process of *eversion* (Striedter and Northcutt 2006) due to the small head size of teleost fish embryos that places stronger spatial constraints on the developing brain (Kotrschal et al. 1990). These findings all point to the possibility that phenotypic integration, both in terms of body size allometry and integration with the skull, could play a major role in constraining the rate and direction of adaptive brain size evolution. However, *if*, *how* and *to what extent* phenotypic integration influences the patterns and processes of brain size diversification in macroevolutionary time scales remain open questions.

Energetic limitation

The brain is one of the most energetically expensive organs in the vertebrate body. In humans for example, brain mass comprises only 2 % of the total body mass but consumes 20 % of the oxygen consumption during resting conditions (Mink et al. 1981). Aiello and Wheeler (1995) showed that the exceptionally large brain in humans and anthropoid primates coincides with a significant reduction in gut size. The expensive tissue hypothesis (ETH)

proposes that energy requirement for increased brain size creates a series of trade-offs between brain size and other energetically expensive organs (Aiello and Wheeler 1995). Moreover, recent findings in relation to the ETH suggest that *any* energetically costly aspects of life, such as reproduction, could coevolve with brain size (the expensive brain hypothesis, EBH) (Isler and van Schaik 2009). ETH and EBH were first proposed as explanations for the extraordinarily large brain size of several anthropoid primates, and subsequent focus on this subject has largely been focusing on mammals and birds. However, recent evidence supports that the energetic limitations on brain size may have significant implications also in ectothermic vertebrates (Kaufman et al. 2003; Kotrschal et al. 2013).

Aims

The goal of my thesis is two-fold. First, I aim to test the generality of the existing ideas that explain mammalian and avian brain size diversity by studying brain size evolution in the most species-rich vertebrate taxon, fishes. Second, I specifically focus on two types of potential constraints on brain size evolution; phenotypic integration and energetic constraints. Accordingly, I study the following topics:

- The role of phenotypic integration in brain size evolution (Paper **I** and **II**).
- The effect of energetic limitation on brain size evolution at a macroevolutionary (Paper **III**) and microevolutionary (Paper **IV**) time scale.
- The adaptive perspective of brain size evolution in relation to ecology, life history, and sexual selection (Paper **V**).

Materials and methods

Study groups

Two groups of fishes were chosen as subject groups in this thesis; Lake Tanganyika cichlids (order Perciformes, family Cichlidae) and pipefishes and seahorses (order Syngnathiformes, family Syngnathidae).

Lake Tanganyika cichlids (family Cichlidae)

The cichlid fishes from Lake Tanganyika in East Africa represents one of the most fascinating and well-studied examples of adaptive radiation (Schluter 2000). Among three separate events of radiations in East African Great Lakes (Lake Malawi, Lake Tanganyika and Lake Victoria), the species flock in Lake Tanganyika, that is composed of approximately 250 species (Salzburger et al. 2005), is the oldest and represents the largest ecological, morphological and genetic diversity (Salzburger et al. 2002). I conducted a field expedition to Lake Tanganyika in 2011 to collect specimens used in my thesis (figure 2). Previous studies on brain evolution in the Lake Tanganyika cichlids have found that algal-eating littoral species have relatively large brains (Huber et al. 1997; Pollen et al. 2007; Gonzalez-Voyer et al. 2009; Gonzalez-Voyer and Kolm 2010), indicating that complex social interactions in the littoral community of the lake (Hori et al. 1993) may have selected for larger brains (Gonzalez-Voyer et al. 2009). Exceptional diversity in morphological, ecological, social, and life history as well as a solid molecular phylogeny in this group (figure 3) offers an appealing opportunity to test various hypotheses on brain size evolution in a phylogenetic comparative framework.

Figure 2. Cichlid community of Lake Tanganyika. Group of *Tropheus moorii* (top) and a male *Cyathopharynx furcifer* with his sand bower (bottom). Photo taken by Alexander Hayward.
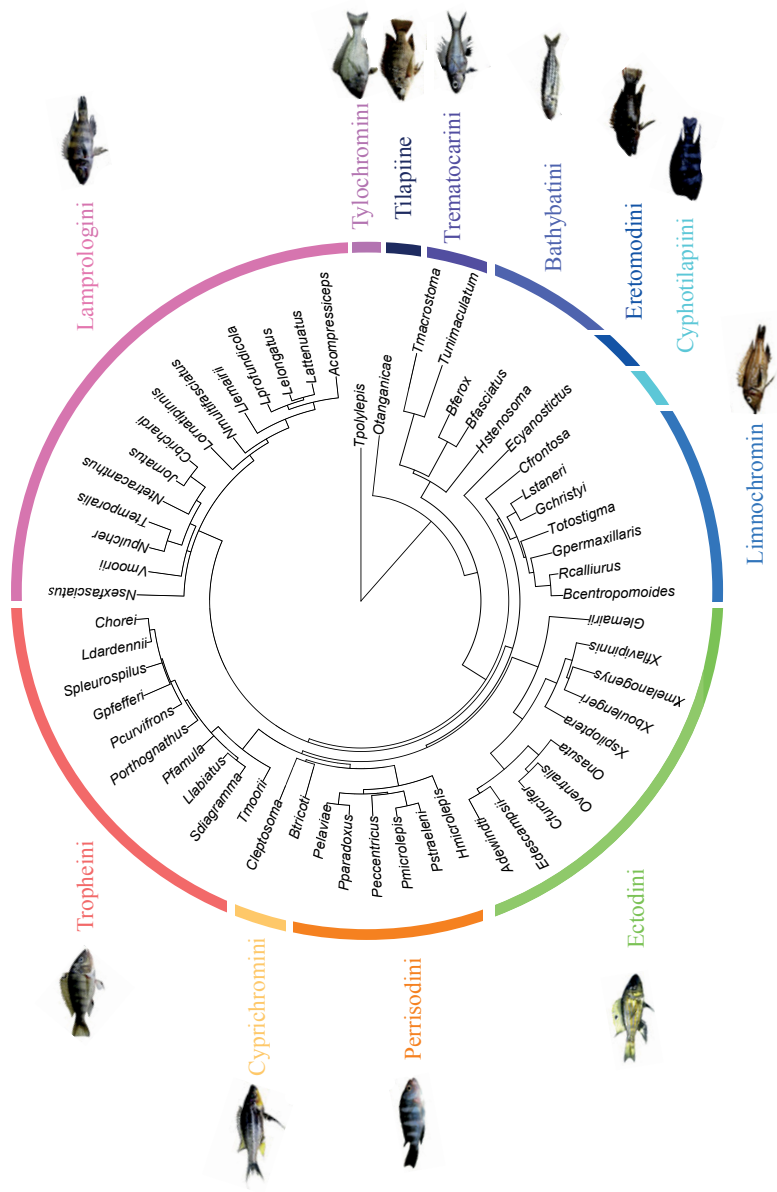
Figure 3. Molecular phylogeny of 57 Lake Tanganyika cichlids used in my thesis. Tribes are represented with different colors and one photo

Pipefishes and seahorses (family Syngnathidae)

The family Syngnathidae consists of about 200 species of pipefishes (Dawson 1985) and 32 species of seahorses (Lourie et al. 1999). This group was chosen to complement two important aspects of variation that do not exist in Lake Tanganyika cichlid; single sex care provisioning by males and stronger sexual selection in females. *Syngnathid* fishes underwent an unrivaled morphological adaptation of the males' brooding structure (Wilson et al. 2003) with which males provide all post-zygotic care of offspring (Berglund et al. 1986b; Wilson et al. 2001; Stolting and Wilson 2007). During copulation, the female transfers unfertilized eggs to the male's ventral surface (figure 4) where fertilization takes place, and males maintain a placenta-like connection that exchange nutrients between embryos and the pregnant males (Sagebakken et al. 2010; Kvarnemo et al. 2011; Goncalves et al. 2015). The family Syngnathidae represent a unique array of variation in the degree and direction of sexual selection, from monogamous seahorses and pipefishes that represent male-male competition as commonly observed across vertebrates (Vincent 1990) to polygynandrous and polyandrous pipefishes where females compete for mating opportunities (Berglund et al. 1986a; Rosenqvist 1990). This extraordinary variation in sexual selection as well as the wide ecological and geographical distribution of the family (Dawson 1985; Foster and Vincent 2004; Lim et al. 2011) provides opportunities to extend the investigation of adaptations and constraints of fish brain size evolution beyond groups with conventional sex roles. I collected samples of pipefishes and seahorses through my own expedition to the Malay Peninsula in 2013, in collaboration with international researchers of Syngnathidae, and through access to museum specimen at National Museum of Nature and Science in Tokyo, Japan. Figure 5 shows a phylogeny of Syngnathidae species studies in my thesis, reconstructed with Bayesian methods based on molecular information and maximum-likelihood estimation based on morphological information.
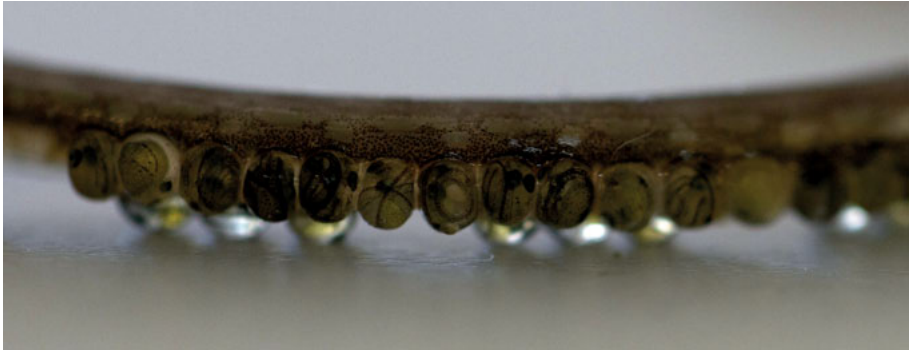
Figure 4 A male pipefishes brooding embryo in their ventral surface. Males of *Nerophis ophidion* (top) and *Syngnathus typhle* (bottom) are shown. Photos taken by Anders Berglund (top) and Ola Jennersten (bottom).
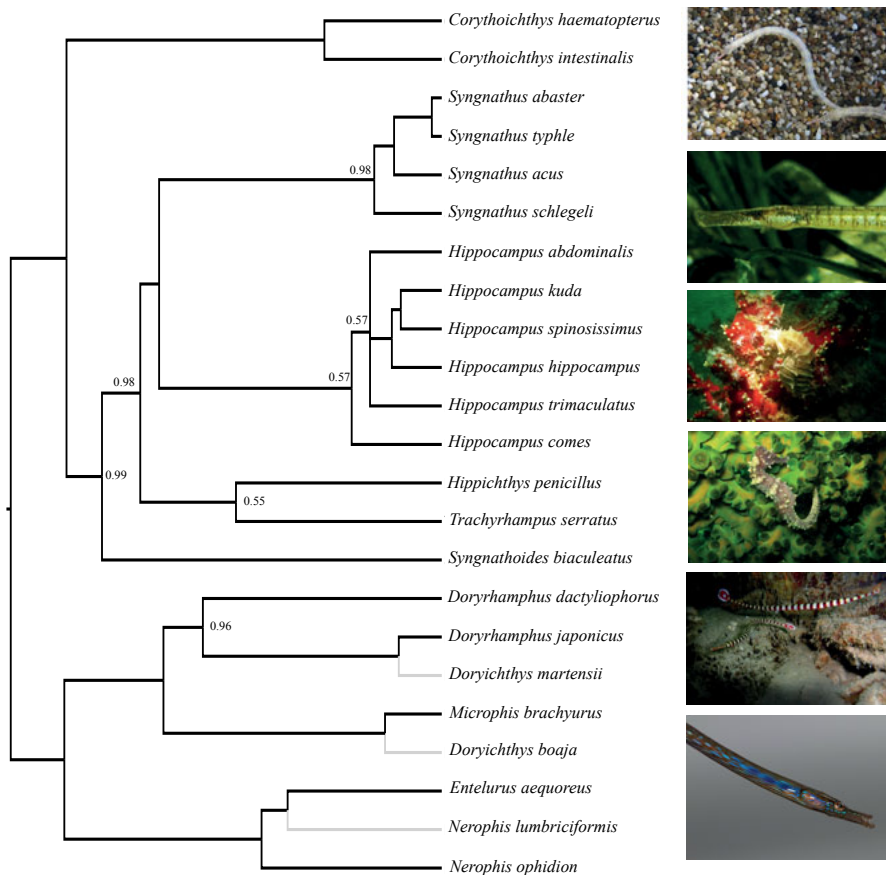
Figure 5. Molecular-morphological phylogeny of 23 species of Syngnathidae based on fragments of three mitochondrial genes, head morphology and brood pouch location. All nodes except for the ones indicated with values have a posterior probability of 1.00. Three grey tips indicate species that were added based on morphological information. Species represented in the photos on the right are (from top to bottom) *Corythoichthys haematopterus* (photo taken by Adam Lim), *Syngnathus typhle* (Anders Berglund), *Hippocampus spinosissimus* (Lau Chai Ming), *Hippocampus kuda* (Lutfi Afiq B. Rosli), *Doryrhamphus dactyliophorus* (Kristen Soong), *Nerophis ophidion* (Anders Berglund).

Phylogenetic comparative analysis

Because species are related according to their history of diversification, species data cannot be treated as independent data points in statistical analysis (Felsenstein 1985). The phylogenetic comparative methods are a series of statistical techniques developed to overcome this lack of independence and are considered a major methodological breakthrough in modern macroevolutionary studies (Harvey and Pagel 1991; Garamszegi 2014). The original application of the phylogenetic comparative method exclusively focused on the removal of phylogenetic signal from species data based on the Brownian motion model of evolution (Felsenstein 1985). However, due to misunderstanding in statistical and biological assumptions behind the application of phylogenetic comparative methods, many studies have misused the comparative methods especially in the context of testing adaptive hypotheses (Hansen 1997, reviewed in Hansen 2014). The core premises of comparative methods in biological context has now began to be recognized again (Hansen 2014) and various approaches that fit alternative evolutionary models are becoming available (Butler and King 2004; Beaulieu et al. 2012; Ingram and Mahler 2013; Pennell et al. 2014; Uyeda and Harmon 2014, reviewed in Garamszegi 2014). In my thesis, I have extensively exploited these conceptual and technical developments in phylogenetic comparative methods. I used a multivariate extension of the conventional correlation analysis with phylogenetic variance co-variance matrix (phylogenetic generalized least square, PGLS; Grafen 1989) (paper **I**). In paper **II**, I took a step forward by testing the rate of evolutionary change rather than the trait value (O'Meara et al. 2006). In paper **III** and **V**, I used the phylogenetic mixed model (Hadfield and Nakagawa 2010) that takes phylogenetic non-independence (Felsenstein 1985) and within species variation (Garamszegi and Moller 2010) simultaneously into consideration. Finally, I analyzed within species variation in brain size within the context of diversification at a macroevolutionary time scale (paper **II** and **IV**). Details of each method are presented in the respective chapters.

Results and discussion

Phenotypic integration of brain size (Paper I)

With their remarkable variation in head morphology (Barel 1983), the Lake Tanganyika cichlid species provide an excellent opportunity to test if phenotypic integration plays a role in brain size evolution. Previous studies on vertebrates have shown that the brain is developmentally integrated with the skull (Richtsmeier and Flaherty 2013), and the skull is developmentally integrated with the jaw (Cooper et al. 2011). Given the tight correspondence between jaw morphology and trophic niche in Lake Tanganyika cichlids (Barel 1983; Clabaut et al. 2007), cognitive adaptation and eco-morphological adaptation may interact indirectly through developmental integration among brain, skull, and jaw. In this paper, I investigated the co-variation patterns between relative brain size (i.e. the brain size after the effect of body size allometry is controlled for), head morphology, and feeding mode to assess if brain-skull-jaw integration exists. To quantify head morphology, we employed geometric morphometrics (figure 6). This technique uses a set of landmarks based on morphological homology across species, and quantifies their variation without decomposing the shape into a subset of length and angle measurements.
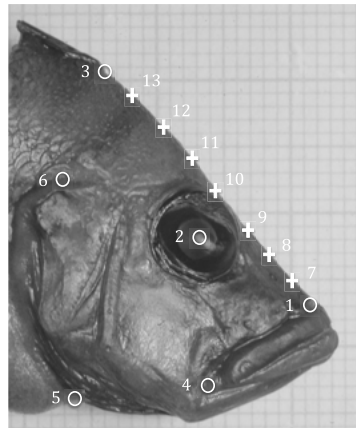

Figure 6. Positions of landmarks used to quantify head shape of Lake Tanganyika cichlids. Homologous landmarks (**O**) and semi-landmarks (+) along the edge of forehead are given. Detailed descriptions for this figure are found in Paper I.

I found that the pre-orbital region of the head, that mainly reflects jaw structure, and the post-orbital region of the head, that mainly represents skull shape, did not form morphologically modular subunits. Subsequently, using PGLS with a multivariate response matrix, I found that both the mode of feeding and relative brain size were significantly associated with head shape (figure 7). These results together suggest that different areas of head regions are morphologically integrated and that two distinctive selective agents; ecomorphological adaptation and cognitive adaptation, may operate simultaneously on head morphology. From this study, I concluded that distinct selection pressures could be linked through phenotypic integration. This may incur a conflict between feeding adaptation and brain size evolution.
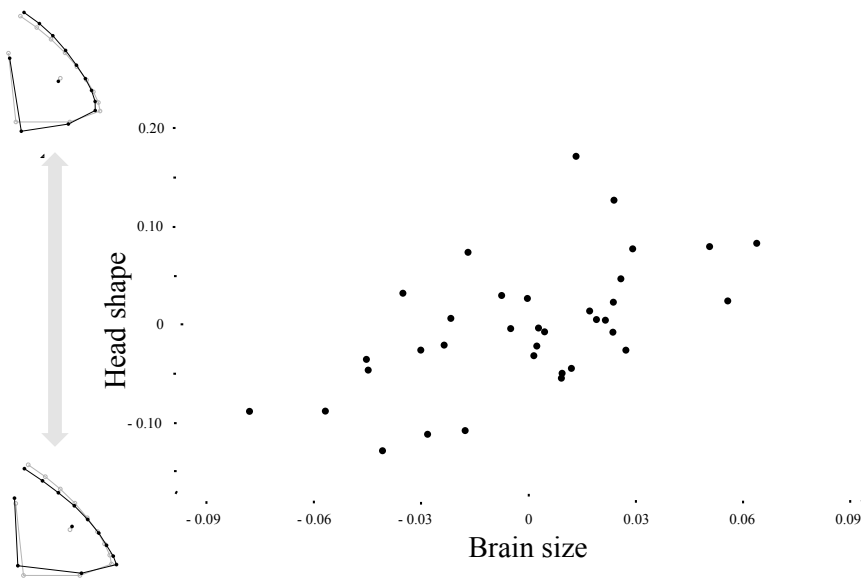

Figure 7. Relationship between relative brain size and head shape in Lake Tanganyika cichlids. Relative brain size is the residual of a phylogenetic generalized least squares regression of log brain weight as a function of log body weight. Y-axis represents the first principal axis of the variation in head shape, and the direction of shape change along this dimension is represented with the black lines connecting closed circles. Average shape is also presented as grey lines connecting open circles.

Evolution of brain-body allometry (Paper II)

In this paper, I extended the investigation of the phenotypic integration in relation to brain size. In paper **II**, I studied the most ubiquitously observed phenotypic integration for brain size: the brain-body static (i.e. within species) allometry (Huxley 1924; Jerison 1973). I employed an analysis of the rate of trait diversification (O'Meara et al. 2006). This analysis first reconstructs the history of character changes using a stochastic simulation (Huelsenbeck et al. 2003), and then compares models that fit a single rate of trait evolution across the entire tree to a multiple-rate model that allows lineages classified differently by stochastic character mapping to have different rates of evolution (O'Meara et al. 2006).

First, I evaluated the patterns of evolution for absolute brain size, relative brain size and static allometry. I found markedly different patterns between these traits. While brain size, both absolute and relative, was characterized by gradual and constant accumulation in trait variance over the history of divergence, static allometry showed a recent rapid diversification. I then tested if static allometry constrains brain size evolution by comparing the performance of various models of evolution that differ in the number of estimated evolutionary parameters. I found no significant evidence that static allometry influence the rate of brain size evolution. Instead, I found that the rate of evolution in static allometry was faster in lineages with small brains and large brains compared to lineages with medium brain size (figure 8). Overall, these findings indicate that brain size evolution may occur mainly along the direction determined by static allometry, supporting the allometric-constraints hypothesis (Voje and Hansen 2012), but that brain size may still evolve relatively independent of body size because of the existing evolvability in static allometry at macroevolutionary time scales.
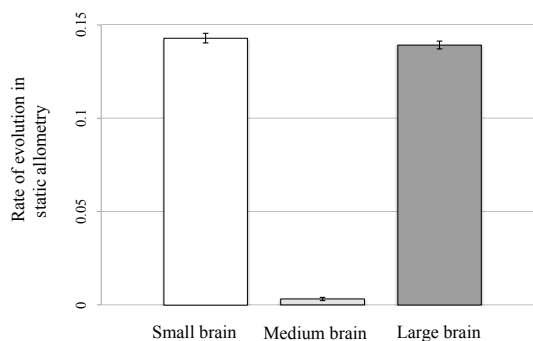

Figure 8. Comparison of evolutionary rate of static allometry between lineages with small, medium and large relative brain size. The presented mean evolutionary rates and standard errors are calculated over 500 phylogenies onto which the transitions in relative brain size were mapped using stochastic simulations.

Revisiting the expensive tissue hypothesis (Paper III)

The brain is one of the most metabolically expensive organs in the vertebrate body (Mink et al. 1981). As a consequence, evolutionary increase in brain size (i.e. encephalization) has been proposed to generate trade-offs with other costly traits to fuel the energetic demands of encephalization (Aiello and Wheeler 1995; Isler and van Schaik 2009). Despite the fact that a number of studies have implied that such energetic limitation is universal across all vertebrates (Kaufman et al. 2003; Kotrschal et al. 2013), surprisingly little attention has been given to the study of exothermic vertebrates in this context. In paper **III**, I investigated the expensive tissue hypothesis and related hypotheses for the first time outside of homoeothermic vertebrates in a macroevolutionary framework. I employed a Bayesian alternative of the conventional PGLS regression (Grafen 1989), the phylogenetic mixed model (Hadfield and Nakagawa 2010). This technique allows analyzing each specimen data point rather than decomposing them into species means, which enables us to account for within species variation.

After controlling for the effect of phylogeny, trophic guild and body size, I found that brain size and gut size were negatively correlated (figure 9). This finding provides the first macroevolutionary support for any ectothermic taxon for the expensive tissue hypothesis in its original form, the brain-gut trade-off (Aiello and Wheeler 1995). Furthermore, egg size and duration of parental care were both positively associated with brain size (figure 9). These results provide support for several variants of energetic constraints hypotheses for brain size evolution. Together with previous findings in other taxa, these results support that energetic limitations are important selection pressures for brain size evolution universally across vertebrates.
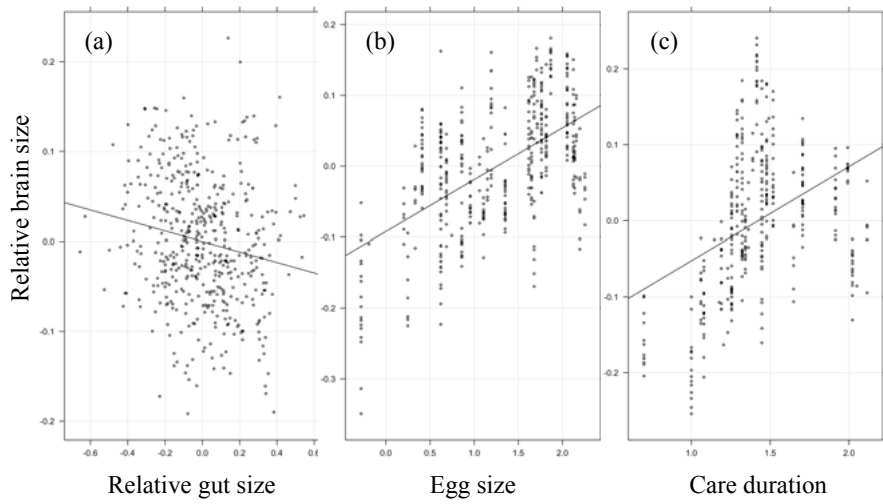
Figure 9. Relationships between relative brain size and relative gut size (a), egg size (b) and care duration (c) of Lake Tanganyika cichlids. Each data point represents single individuals and an ordinary least square regression is provided.

The expensive tissue hypothesis within species (Paper IV)

An emerging research field in the evolutionary ecology of brain morphology is the investigation of variation in brain size at within species (microevolutionary) levels (Gonda et al. 2013). In this paper, I investigated the expensive tissue hypothesis using within species variation of one species of pipefish, *Syngnathus schlegeli*. Like in all other *Syngnathus* species, male *S. schlegeli* provide all post zygotic care of offspring in a brood pouch (Watanabe and Watanabe 2001). The reproductive ecology of this species offers interesting contrasts in reproductive states that can be used to test the ETH at the within species level (i.e. brooding males, non-brooding males and females). The relative size of the brain and other energetically expensive organs (kidney, liver, heart, gut, visceral fat, ovary/testis) was compared among these groups.

Brood size in pregnant males was unrelated to brain size or the size of any other organ, whereas positive relationships were found for ovary size with kidney and liver size in females. Moreover, we found that the size of a suite of energetically expensive organs (brain, heart, gut, kidney, liver) as well as the amount of visceral fat did not differ between pregnant and non-pregnant males. However, we found marked differences in the relative size of the expensive organs between sexes. Females had larger liver and kidney than males, whereas males stored more visceral fat than females (figure 10). Furthermore, in females we found a negative correlation between brain size and the amount of visceral fat (figure 11), whereas in males a positive trend between brain size and both liver and heart size was found. These results suggest that, while the majority of variation in the size of various expensive organs in this species likely reflects that individuals in good condition can afford to allocate resources to several organs (van Noordwijk and de Jong 1986), the cost of the expensive brain was visible in the visceral fat content of females, possibly due to the high costs associated with female egg production. This study indicates that patterns seen at microevolutionary scales may be linked to patterns at macroevolutionary time scales.
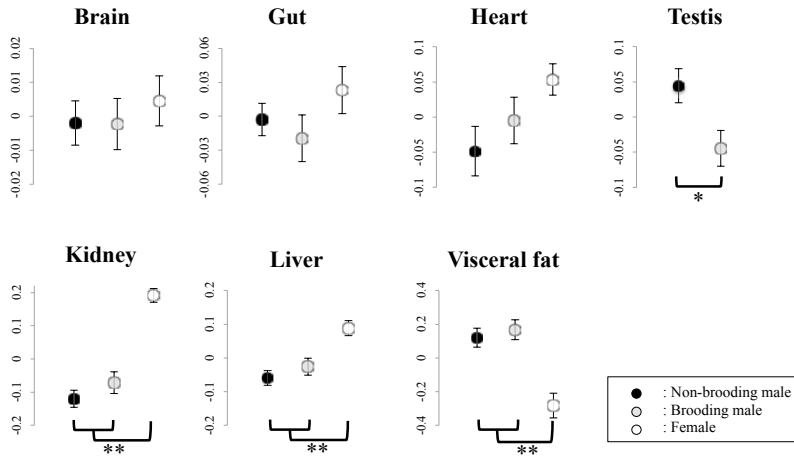
Figure 10. Comparison of relative organ size between non-brooding males, brooding males and females of *S. schlegeli*. Relative size of brain, gut, heart, testis, kidney, liver and visceral fat are residuals of an ordinary least squares regression of $\log_{10}$ organ/fat size against $\log_{10}$ body length.
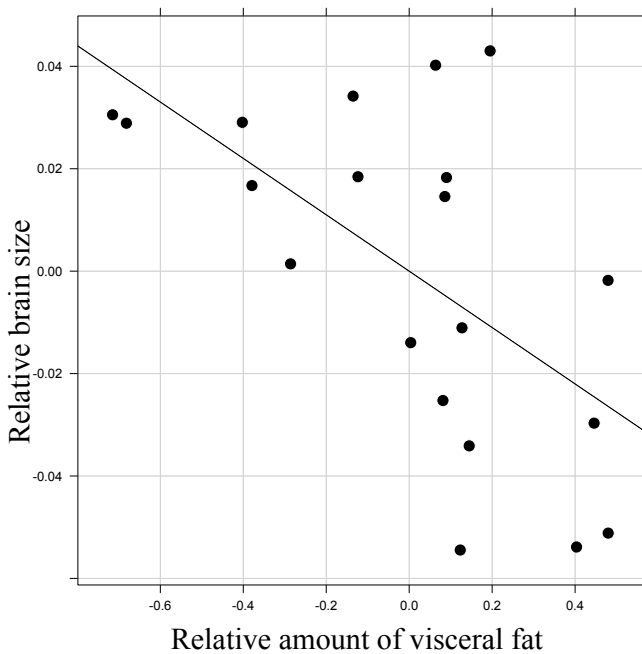

Figure 11. Relationship between relative brain size and relative amount of visceral fat in female *S. schlegeli*. A significant negative relationship was found (r ± s.e. = - 0.48 ± 0.18, p = 0.008).

Brain size evolution in Syngnathidae (Paper V)

One of the limitations in previous studies of vertebrate brain size evolution was their almost exclusive focus on species in which males compete for the opportunities for mating and females choose mates (i.e. conventional sex roles). In paper **V**, using the Syngnathid fishes that have undergone a dramatic diversification in sex roles (Vincent et al. 1992), I investigated the role of ecology, life history and sexual selection in relation to relative brain size in this group where females are generally under stronger sexual selection than males. For these analyses, I collected a data set of brain size for 339 individuals across 23 species of pipefishes and seahorses and the data was analyzed with the phylogenetic mixed model (Hadfield and Nakagawa 2010).

Table 1 summarizes the result of a phylogenetic mixed model. I found that relative snout length (i.e. snout length compared to head length (SnL/HL)) and egg size were both positively associated with relative brain size. Additionally, I found a female favored sexual brain size dimorphism. Female syngnathids had on average 4.3 % heavier brains than males of the same species and the same body size. Interestingly, female favored sexual brain size dimorphism was more pronounced in polygynandrous species than in monogamous species (fig 12). These results show that feeding adaptation for motile prey items, energetic constraints associated with offspring production and female mating competition are associated with variation in brain size in pipefishes and seahorses.

Table 1. Summary of phylogenetic mixed model that tested correlations between ecology, life history and sexual selection and relative brain size while taking the phylogeny into account. Body length is included as a covariate in all models.

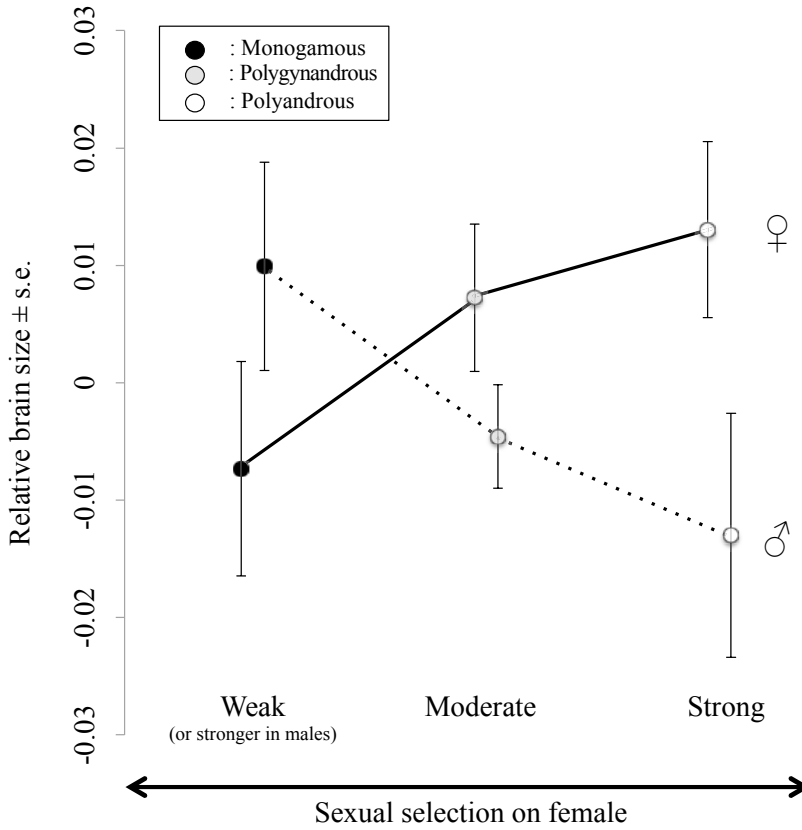| Response	Main predictor	Posterior mean [95% C.I.]	p
Brain size	SnL/HL ratio	$0.921_{[0.367, 1.519]}$	**0.002**
	Mating pattern	$-0.097_{[-0.300, 0.120]}$	0.341
	Egg size	$0.136_{[0.014, 0.267]}$	**0.036**
	Brood size	$0.056_{[-0.094, 0.202]}$	0.436
	Brood duration	$0.011_{[-0.007, 0.028]}$	0.213
	Sex	$-0.019_{[-0.032, -0.004]}$	**0.013**
	Mating pattern: Sex	$-0.02_{[-0.04, -0.001]}$	0.053

Figure 12. Visualization of the interaction between mating pattern and sex on brain size. Y-axis indicate relative brain size (i.e. residuals of ordinary least squares regression of $\log_{10}$ brain weight as a function of $\log_{10}$ body weight) and X-axis indicate the strength of sexual selection in females which is estimated from the mating pattern as follows; monogamy: weak sexual selection (black circle), polygynandry: moderate sexual selection (grey circle), polyandry: strong sexual selection (open circle). Females are connected with solid lines and males are connected with dashed lines.

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Summary and future remarks

Bran size evolution in cichlids, pipefishes and seahorses

In the present thesis I have investigated a range of hypotheses in relation to brain size evolution using two diverse families of fish, Cichlidae and Syngnathidae. I have demonstrated i) that phenotypic integration can link functionally unrelated traits, and this may constrain independent evolution of each part involved or promote concerted evolution of an integrated whole, ii) that brain-body static allometry constrains the direction of brain size evolution, even though the static-allometry showed ability to evolve, allowing evolution of relative brain size under allometric constraints, iii) that the energetic constraints of development and maintenance of brain tissue is an important factor in forming the diversity in brain size in cichlids and syngnathids, both at macroevolutionary and microevolutionary time scales, and iv) that adaptation for feeding and female mating competition may have played key roles in the adaptive evolution of brain size in pipefishes and seahorses. Below, I will summarize the four most important aspects involved in brain evolution in cichlids, pipefishes and seahorses; feeding ecology, sexual selection, phenotypic integration and energetic constraints.

Feeding ecology
Feeding ecology is evolutionarily associated with brain size across many vertebrate taxa (Striedter 2005). For example, consumption of food items that are cognitively challenging to find or catch (Cluttonbrock and Harvey 1980; Hutcheon et al. 2002), wide diet breadth (MacLean et al. 2014) and propensity for innovative food acquiring behavior (Nicolakakis and Lefebvre 2000; Garamszegi et al. 2005) are strong predictors of large brain size across species. These studies indicate that selection for the ability to explore and exploit food resources may be a common underlying force for adaptive brain size evolution (Striedter 2005; MacLean et al. 2014). In my thesis, I found that pipefishes and seahorses that consume mobile prey such as mysids, shrimps and fish have larger brains than species that prey on slow moving prey. Given that the cognitive ability required to in search for and catch evasive prey is presumably higher than that required to catch slow moving prey

items, the found pattern supports the idea that feeding adaptations select for increased brain size in Syngnathidae.

Sexual selection
Since Darwin, it has been an enduring idea that mate choice could be directly based on the cognitive ability of potential partners (Miller 2000; Boogert et al. 2011) and that intra-sexual competition over mating opportunities often involves cognitive challenges (Francis 1995; Jacobs 1996; Boogert et al. 2011). Using the family Syngnathidae, in which sexual selection is generally stronger in females than in males, I showed that females represent 4.6 % heavier brains than males of the same body size. Moreover, the degree of female-favored brain size dimorphism is more pronounced in polyandrous species than in monogamous species (figure 12). A few studies have previously reported male-favored brain size dimorphism in species where males compete for mates (Iwaniuk 2001; Kotrschal et al. 2012). Although these studies have claimed that the pattern would be in line with the hypothesis that stronger sexual selection within males lead to large male brain size, they were not able to disentangle the effect of numerous other variables that commonly co-vary with sex. By demonstrating paralleling patterns in females that experience strong sexual selection, my study provides general support for the idea that sexual selection affects brain evolution.

Phenotypic integration
It has long been discussed that the correlation between brain size and other traits could serve as a constraint for adaptive brain size evolution (Jerison 1973; Lande 1979; Striedter and Northcutt 2006). My thesis provided two of the first direct investigations to this old hypothesis with a rigorous phylogenetic comparative approach. I found covariation between brain size, head morphology and feeding mode as well as limited modularity in cichlid head morphology. These results indicate an interesting possibility that cognitive adaptation and trophic adaptation can be linked through phenotypic integration. As a result, independent evolution of brain size may be restricted. Such constructional constraints (Gould and Lewontin 1979) have been proposed to explain the concerted evolution of increased brain size and decreased jaw size during hominid evolution (Stedman et al. 2004). Our results propose that this type of constraint might not be limited to large brained anthropoid primates.

   In my investigation of the evolution of within species (static) allometry, I found somewhat contradicting results to this finding. I found a trend for allometric constraints on brain size evolution. There was i) a tendency that strong static allometry constrains the rate of relative brain size evolution, and ii) support for stabilizing selection for static allometry to follow a common allometric slope. This follows the prediction of the allometric-constraints hypothesis (Voje et al. 2013). However, I also found that static allometry has

the ability to evolve over long time scales (i.e. millions of years) and that the rate of evolution of static allometry differs between groups of different brain size. Based on these results, I propose that brain evolution is partially constrained but not completely governed by static allometry if directional selection for change in brain size is strong enough. To sum up, although it appears to be true that the constant growth regulation does constrain the trajectory of brain size evolution (Huxley 1924; Jerison 1973; Lande 1979), the ability of static allometry to evolve appears to be large enough to cross the boundary of developmental regulation mechanisms, enabling brain size to evolve relatively independent of the influence of allometric constraints.

Energetic constraints
The energetic cost of increased brain size was first hypothesized to explain hominid evolution (Aiello and Wheeler 1995) and subsequent attention has primarily focused on large-brained, homeothermic animals. However, a growing number of studies now demonstrate that energetic limitations on brain size is not a particular constraint for large brained animals but universally applicable across vertebrates including ectothermic vertebrates (Kotrschal et al. 2013; Iglesias et al. 2015). My thesis provide several important results that render support for and extend the idea that energetic constraints are important factors in forming the diversity of vertebrate brain size. Particularly, I found that egg size was a strong predictor of variation in brain size in both Cichlidae and Syngnathidae. Larger brains were associated with larger eggs in both cases, results that support those found in placental mammals (Isler and van Schaik 2009) and birds (Isler 2011). Furthermore, in cichlids, duration of parental care was positively correlated with brain size, which fits with the prolonged gestation/parental care period found among large brained homeothermic species (Iwaniuk and Nelson 2003; Isler and van Schaik 2006; Isler and van Schaik 2009; Isler 2011). I also demonstrated a negative correlation between brain size and the amount of visceral fat storage in the Pacific seaweed pipefish. This is the first micro evolutionary support for a recent finding of a negative association between brain size and adipose tissue in primates (Navarrete et al. 2011).

Future remarks

Microevolution and Macroevolution

"The crucial point in population differentiation is that at which discontinuity appears between parts of what was previously a continuous population. ... The most important difference of opinion, at present, is between those who believe that discontinuity arises by intensification or combination of the differentiating processes already effective within a potentially or really continuous population and those who maintain that some essentially different factors are involved. ... Micro-evolution involves mainly changes within potentially continuous populations, and there is little doubt that its materials are those revealed by genetic experimentation. Macro-evolution involves the rise and divergence of discontinuous groups, and it is still debatable whether it differs in kind or only in degree from micro-evolution. If the two proved to be basically different, the innumerable studies of micro-evolution would become relatively unimportant and would have minor value in the study of evolution as a whole."

George G. Simpson 1944 in Tempo and Mode in Evolution

This paragraph written by G. G. Simpson in 1944 captures what we can learn from the studies summarized in my thesis. It is, by the way, striking that what was mentioned as the most important difference of opinion "at present" more than seven decades ago is probably still one of the fundamentally different schools-of-thought in evolutionary biology today. We are yet to agree on if or how micro- and macroevolutionary processes are linked.

One of the challenges that I faced in my thesis was to bridge patterns of variation observed at *within* species (microevolutionary) levels to patterns of variation observed at *between* species (macroevolutionary) levels. This was particularly relevant to paper II and paper IV. These two studies both demonstrated that microevolutionary patterns and macroevolutionary patterns could be comparable if one is cautious concerning the significant differences in time scales and to the precise models of evolution. Importantly, if microevolution and macroevolution were generally compatible with each other, one can predict macroevolutionary outcomes based on microevolutionary patterns and vice versa. Major theoretical and methodological advances have already been made in this area of research (Steppan et al. 2002;

Hansen and Houle 2008). I believe that future studies with more rigorous microevolutionary approaches, especially in the fields of quantitative genetics and genomics, and employment of more elaborate and biologically realistic evolutionary models in phylogenetic comparative methods will be a key to unify microevolution and macroevolution.

Phylogenetic comparative analyses

With regard to a specific methodological detail of phylogenetic comparative methods, there is one important path for further improvement. In my thesis, I have mostly employed various versions of Brownian motion as the model for trait evolution. Due to its simplicity, this model is very easy to handle and interpret. However, real evolutionary processes may not always follow such a simple random walk processes (reviewed in Pennell and Harmon 2013). There are several alternative models of evolution to consider in a biological context; the Early-Burst model (Harmon et al. 2010), the Ornstein-Uhlenbeck model with or without multiple optima and rate changes (Hansen 1997; Butler and King 2004; Beaulieu et al. 2012), the Acceleration-Deceleration model (Blomberg et al. 2003) and early-burst model (Harmon et al. 2010), Speciation- and Diversity-based models (FitzJohn 2010; Magnuson-Ford and Otto 2012) and various combinations of these. Attempts to incorporate these alternative models have just started to develop (Pennell and Harmon 2013; Garamszegi 2014; Uyeda and Harmon 2014; Pennell et al. 2015) and consideration of these alternative evolutionary models would lead to better understanding of the data presented in my thesis.

Brain size diversification across teleost fishes

One approach to further investigate brain size evolution in fish is to expand the range of studies outside of the two groups studied in my thesis. Figure 13 shows the brain-body allometry of 489 marine teleost species, collected from Iglesias et al. (2015), compared to that of cichlids and syngnathids used in my thesis. Interestingly, the family Cichlidae and Syngnathidae have both underwent a significant reduction in relative brain size compared to many marine teleost fishes. Why do these two groups have on average smaller relative brains? Is this difference a result of reductions in brain size, or increases in body size without accompanying change in brain size, or both? Or do the other marine species in this comparison live in physical and social environments with higher cognitive demands? These questions could be answered by expanding the investigation of brain size to many more groups of fishes in addition to cichlids and syngnathids.
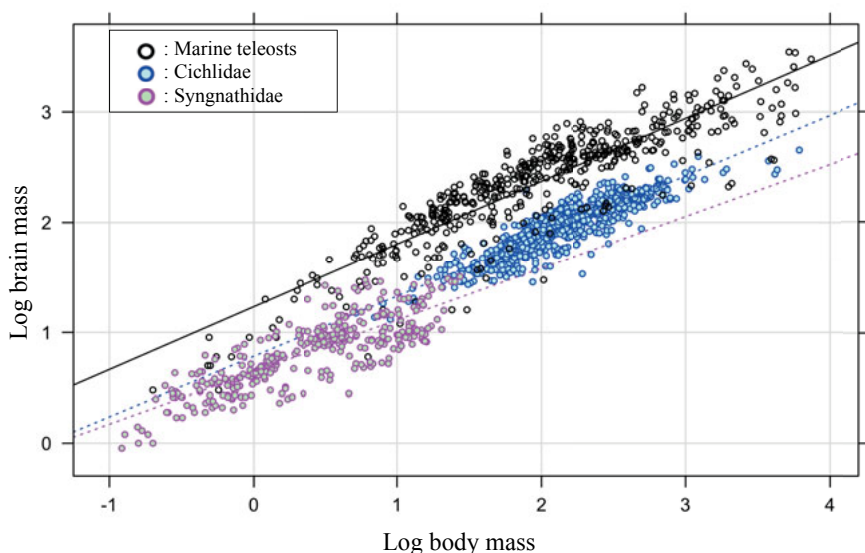
Figure 13. Relationship between log-brain size and log-body size of 489 species of marine teleost fish (open circle), 71 species of cichlids (blue circles) and 23 species of pipefishes/seahorses (magenta circles). Ordinary least squares regressions are provided for marine teleost (solid black line), cichlids (dashed blue line) and pipefishes/seahorses (dashed magenta line) separately.

Brain structures

Finally, it is worth noting that my thesis is entirely written about the evolution of brain size. Given the generally high predictability of structural size from the overall brain size (Finlay and Darlington 1995, but see Iwaniuk et al. 2004; Noreikiene et al. 2015) my approach should be justified as long as the investigation is related to brain properties that are shared between structural parts. This would be the case for phenotypic integration and energetic constraints, but caution should be taken when adaptive explanations for variation in brain size are given (Healy and Rowe 2007). It will be important to investigate also the existing variation in brain region size, neuron density and connectivity (Striedter 2005; Herculano-Houzel 2012) to provide a more complete picture of how ecology, life history, behavior and sexual selection shape brain morphology among contemporary vertebrates.

Conclusions

To conclude, my thesis proposes that feeding ecology, sexual selection and energetic constraints are three key factors involved in forming the contemporary diversity of brain size in the family Cichlidae and Syngnathidae. On the other hand, although the thesis demonstrated a clear involvement of phenotypic integration in brain size diversification, the available data presented in my thesis imply that this type of constraints may have limited effects on brain size evolution at long-term macroevolutionary time scales.

My thesis exploited the wide range of variation in ecology, life history, and sexual selection represented by these two fascinating families of fish to provide critical and novel insights in our understanding of macroevolutionary patterns and processes of brain size diversification. Moreover, by employing a number of state-of-the-art phylogenetic comparative methods, my thesis investigated novel types of questions as well as old questions in novel ways. In summary, my thesis shows that phylogenetic comparative analysis using fishes as a model system can be a rewarding avenue of research for the study of vertebrate brain size evolution.

Sammanfattning på svenska

Bakgrund

Hjärnans storlek är extremt variabel bland däggdjuren (Jerison 1973) och att förklara denna variation har varit ett viktigt fält inom evolutionsbiologin ända sedan Darwins första texter i ämnet (Darwin 1871). Den övergripande förklaringsmodellen för de processer som genererat variation i hjärnstorlek är baserad på antagandet att hjärnan evolverar genom en balansering av selektion för ökad kognitiv förmåga och energikostnaderna som följer av att utveckla en större hjärna och att försörja detta kostsamma organ med nödvändiga energiresurser (Aiello & Wheeler 1995). Tidigare studier på hur anpassningar och begränsningar har påverkat vertebrathjärnans evolution har fokuserat mestadels på däggdjur och fåglar. Jag har istället studerat hur anpassningar och begränsningar påverkat hjärnans evolution hos den mest variabla djurgruppen på jorden, fiskarna. Mer specifikt så har jag använt ciklider och kantnålar och sjöhästar, två extremt diversa fiskfamiljer som modellsystem för mina studier.

Material och metoder

<u>Modellsystem</u>
Ciklidfiskarna som finns i Tanganyikasjön i Afrika representerar ett av de mest fascinerande och välstuderade exemplen på adaptiv radiation (Schluter 2000). Gruppen består av ca 250 arter som evolverat på mycket kort tid och som uppvisar extrem ekologisk, morfologisk och genetisk variation mellan de olika arterna (Salzburger et al. 2002).

Familjen Syngnathidae, kantnålar och sjöhästar, består av ca 200 arter (Dawson 1985) och uppvisar även den stor variation i beteenden och morfologi. Till skillnad från cikliderna och de flesta andra djurgrupper, så spenderar hannar mycket mer resurser än honor på att ta hand om ägg och avkomma hos många arter i denna grupp (Berglund et al. 1986b; Wilson et al. 2001; Stolting and Wilson 2007). Det finns vidare stor variation i nivåerna av sexuell selektion på honor respektive hannar hos kantnålar och sjöhästar (Vincent 1990; Berglund et al. 1986a; Rosenqvist 1990). Detta gör att gruppen är ett mycket lämpligt komplement till andra grupper för att studera länken mellan sexuell selektion och könsskillnader i hjärnans storlek och för att testa generella idéer som bäst studeras hos arter med stor variation i könsroller.

<u>Phylogenetiska jämförande analyser</u>

Eftersom arter är besläktade genom deras släkträd (fylogeni) kan separata arter inte anses vara oberoende datapunkter (Felsenstein 1985). Så kallade jämförande fylogenetiska analyser krävs därför vid mellanartsjämförelser för att kontrollera för dylika släktskap (Harvey & Pagel 1991; Garamszegi 2014). Man kan dessutom använda moderna varianter av dessa metoder för att studera evolutionära skeenden i fylogenier. I min avhandling har jag använt de senast utvecklade analysmetoderna inom denna metodik och analyserat enkla mellanartskorrelationer (Grafen 1989), mellanartskorrelationer som tar hänsyn till inomartsvariation (Hadfield & Nakagawa 2010), evolutionshastigheter (O'Meara et al. 2006), och evolutionen av inomartsvariation (Hadfield & Nakagawa 2010; Garamszegi & Möller 2010).

Resultat

- Hjärnstorlek och huvudmorfologi formar en fenotypiskt integrerad enhet som kan påverka hjärnans evolution (**artikel 1**).

- Allometri mellan hjärnans storlek och kroppsstorlek har påverkat hjärnans evolution hos ciklidfiskarna men hjärnans storlek har trots detta evolverat relativt oberoende hos denna grupp (**artikel 2**).

- De höga energikostnader som stora hjärnor kräver har påverkat andra organ. Hos cikliderna var stora hjärnor associarade med mindre mag-tarm paket och större ägg medan äggstorleken var större hos arter med stora hjärnor hos kantnålar och sjöhästar och fettreserverna var mindre hos honor med stora hjärnor hos en kantnålsart (**artiklar 3, 4 och 5**).

- Anpassningar till att äta snabbt simmande byten är positivt associerade med större hjärnor hos kantnålar och sjöhästar (**artikel 5**).

- Sexuell selektion, framförallt hos honor, är starkt positivt associerad till hjärnans storlek hos kantnålar och sjöhästar (**artikel 5**).

Diskussion

Baserad på moderna analyser av data på både inom- och mellanartsnivå har jag i min avhandling testat flera viktiga hypoteser rörande hur hjärnan evolverar bland ryggradsdjuren. Jag har i mina analyser funnit stöd för att hjärnan påverkas av kroppsmorfologi både vad gäller huvudets form och generell kroppsstorlek och därmed demonstrerat dessa samband för första gången med storskaliga fylogenetiska analyser hos växelvarma djur. Vidare så fann jag att sambandet mellan kroppsstorlek och hjärnstorlek spelar roll för riktningen av hjärnans evolution, men även att förhållandet mellan kropp och hjärna kan förändras över långa evolutionära tidsperspektiv. Jag fann starka indikationer på att investeringar i hjärnans storlek måste balanseras mot andra kostsamma organ såsom mag-tarm paketet och fettreserver. Jag fann även att arter med större hjärnor lägger större ägg, som troligen leder till större ungar. Slutligen så fann jag att anpassningar till att äta snabbt simmande bytesdjur och graden av sexuell selektion, framförallt hos honor, är starkt länkade till större hjärnstorlek hos kantnålar och sjöhästar. Min avhandling har därmed genererat viktiga generella data som stödjer flera, men inte alla, viktiga hypoteser gällande hjärnans evolution hos ryggradsdjuren. Min avhandling har därmed ökat vår förståelse för hur variationen i hjärnstorlek evolverat hos olika djurgrupper. Vidare så visar min avhandling, i egenskap av kombinationen av inomartsanalyser och mellanartsanalyser, att det går att kombinera olika evolutionära nivåer för att öka vår förståelse över hur egenskaper evolverar både inom och mellan arter.

日本語要約

カワスズメ科・ヨウジウオ科魚類における脳サイズ進化の系統的種間比較研究

緒言

脊椎動物の脳は神経系の中枢として生命活動の中心的・指導的役割を担う器官である。それ故、脳の進化は古くから進化生物学の重要研究課題として注目され、現在まで膨大な研究成果が蓄積されてきた (Jerison 1973; Striedter 2005)。様々な研究アプローチの中でも、相対脳サイズ（脳サイズの変異から体サイズの影響を除去した変数、以下脳サイズとする）の種間比較研究は脳サイズの適応進化にかかる選択圧の特定に大きく貢献した。例えば大きな脳サイズは複雑な認知能力を必要とする食性への適応 (MacLean et al. 2014)、高度な社会性 (Barton et al. 1996; Dunbar and Shultz 2007)、行動の柔軟性 (Lefebvre et al. 1997; Nicolakakis and Lefebvre 2000; Sol and Lefebvre 2000; Timmermans et al. 2000; Lefebvre et al. 2002)等と関連していることから (reviewed in Striedter 2005)、脳サイズの進化的増加は認知能力向上が利益となる環境において生じることが示唆されてきた。他方で、脳は成長と維持に膨大なエネルギーを消費する器官である (Aiello and Wheeler 1995)。そのため、脳サイズの増加は進化の過程において様々なエネルギー収支上のトレードオフと伴って生じてきた。さらに、脊椎動物の脳が収納されている頭部のサイズは生物の構造上体サイズと比較して不釣り合いに巨大化することが不可能であることから、脳サイズの進化は顎部のサイズとの構造的トレードオフ関係を保ちながら増大・衰萎してきた (Holekamp et al. 2013)。総じて、脳サイズは適応とエネルギー的・構造的制約のバランスの中で進化してきたことがこれまでの脳サイズ種間比較研究から明らかとなってきた。

　歴史的に脳サイズ進化の研究はなぜヒト *Homo sapiens* が類人猿を含む霊長類と比較して大きな脳サイズを持っているのか、という問いから始まった (Huxley 1863)。そのため、脳サイズ進化の研究がこれまで主に霊長類と霊長類を含む哺乳類を対象分類群としてきたことは不思議ではない。しかし、哺乳類は脊椎動物全体の１０％を占めるに過ぎないため、脊椎動物全般に普遍的な脳サイズ進化のメカニズムを特定する事はこれまでの研究では不可能であった。特に脊椎動物の大多数を占める変温動物は脳サイズの研究ではほとんど着目されて来なかった。そこで本研究では種数の上で脊椎動物の多数を占め、様々な環境に適応してきた魚類を対象に脳サイズ進化を研究することで、これまで限られた分類群間で議論されてきた諸仮説の普遍性を評価すること、そして新たな仮説を提唱することを目的とした。

材料と方法

カワスズメ科 Cichlidae とヨウジウオ科 Syngnathidae
アフリカ東部の大地溝帯に位置する巨大な淡水湖：タンガニイカ湖に生息するカワスズメ科魚類は適応放散の教科書的例として知られ、野外における進化生物学のモデル系である (Schluter 2000)。カワスズメ科魚類はタンガニイカ湖のあらゆる生物的・非生物的環境に適応して劇的な種分化を遂げ、現在記載されている約２００種の形態・生態・生活史・行動的多様性は単一科内で見られる多様性としては他に類いを見ないほどに大きい (Salzburger et al. 2002)。本分類群における脳サイズ進化に関しては、先行研究で浅瀬に生息する藻類食者（主にトロフェウス族）が他のカワスズメ科よりも大きな脳サイズを持っていることが報告され、トロフェウス族の生息環境で特異的に見られる複雑な種内・種間相互作用がタンガニイカ湖産カワスズメ科魚類の脳サイズ進化に主要な役割を持つことが議論されてきた (Gonzalez-Voyer et al. 2009)。

　ヨウジウオ科は熱帯から寒帯、淡水から海水まで世界中のあらゆる水域に生息する分類群で形態の大きく異なる２属：タツノオトシゴ属（約３２種）とヨウジウオ属（約２００種）から構成される (Dawson 1985; Lourie et al. 1999)。本分類群はメスがオスの育児嚢に卵を産卵し、オスが「妊娠」するという極めて特異な養育方式を進化させた分類群として有名である (Berglund et al. 1986b; Wilson et al. 2001; Stolting and Wilson 2007)。この特殊な生殖生態と伴い、ヨウジウオ科魚類では一般的にメスがオスとの繁殖機会を巡って競争し、オスが繁殖相手としてメスを精査する。これは有性生殖を行う大多数の生物とは逆のパターンであり、行動生態学において性淘汰の検証を行うモデル系として主に研究されてきた (Berglund et al. 1986a; Rosenqvist 1990; Ahnesjo and Craig 2011)。本研究においても性淘汰と脳サイズの関連を検証することを主な目的として対象分類群に選定した。

系統的種間比較法
種は分化してきた歴史の糸（系統樹）によって繋がれているため、近縁な種同士が類似した形質が、遠縁な種同士では相違した形質が一般的に観測される。その結果、種間のデータを統計学的に独立なデータ点として扱う事はできない (Felsenstein 1985)。系統的種間比較法はこの問題提起から発達した種々の統計的手法の総称である (Garamszegi 2014)。本研究課題において、統計的種間比較法は統計的に妥当な分析を行うために必要不可欠な手法であるため全編を通じて使用した。また、形質の進化モデルを系統樹情報と現存する形質値の種間変異に基づいて推定する最新の分析方法も用いた。

結果
- 脳サイズと頭部形態は進化的に統合されていることがわかった（**論文1**）
- 脳と体の相対成長（アロメトリー）によって脳サイズ進化の方向が制約されていることが示された。一方でアロメトリーそのものも１００万年単位の時間尺度では進化していた（**論文2**）
- 脳サイズと腸サイズ（**論文3**）、内臓脂肪貯蔵量（**論文4**）の間にトレードオフが見られた
- 脳サイズの増加に伴い卵サイズの増加と養育期間延長が見られた（**論文3**、**論文5**）
- 脳サイズと相対口吻長と相関していた（**論文5**）
- メスにかかる性選択の強い種ではメスの脳サイズがオスと比較して大きい性的二型が見られた（**論文5**）

考察とまとめ

本博士論文ではカワスズメ科魚類とヨウジウオ科魚類の形態・生態・生活史の多様性に着目し、脊椎動物の脳進化にかかる適応と制約について系統的種間比較法を用いて研究を行った。その結果、１）採餌に関する認知能力、２）脳の発達と維持にかかるエネルギー上の制約、３）体サイズ・頭部形態との形態的統合による制約、そして４）生殖を巡る競争に必要な認知能力が対象分類群における脳進化に重要な役割を果たしたことが示された。これらの結果は哺乳動物と鳥類で主に研究されて来た従来の仮説に変温動物を用いた新たな検証例を提示することで脊椎動物全般に一般性を与えた。加えて、本研究ではこれまで研究が進んでこなかった研究課題に関する新たな知見を得ることもできた。すなわち、性的役割の逆転したヨウジウオ科で見られたメスに偏った性的脳サイズ二型は、脳サイズが性淘汰によって進化している可能性を支持していた。さらに、アロメトリーの進化と脳サイズ進化の関連については、古くから理論的なアイデアは提案されていたものの (Huxley 1932; Gould and Lewontin 1979)検証方法が発達していなかった。本研究では最新の系統的種間比較法を駆使することでこの古い問題に新たしい答えを提案することに成功した。

　最後に、本研究を遂行する上で最も苦心した課題は小進化と大進化の隔たりであった。上述の通り本研究は系統的種間比較法の発展を駆使し、主に種間変異を対象に行われた。そのため、全体を通じて本研究は種間以上のスケール、一般的に種が分化する１００万年単位以上の時間尺度における形質の進化パターンについての研究である。これと対照的なアプローチが種内の変異パターンについて実験的に研究する手法で、これら種内における小進化の研究は一般的に数世代から数十世代にわたる世代交代時間の中で生じる進化パターンを対象としている。ここで生じる本質的な疑問は、これら大進化と小進化が異なっているのは単純に時間スケールであって小進化のプロセスを１００万年単位に引き延ばすことで大進化のプロセスは予測できるのか、

それともこれら二つの時間軸における進化には本質的な違いがあるのか、という点である（Simpson 1944）。本研究ではこの問題提起を踏まえ、二つの点で概念的に新たな取り組みを行った。まず論文 2 では、種内で見られる形質変異に基づいて脳サイズと体サイズの形態統合の強さを評価し、それを大進化の時間スケールで解析した。次に論文 4 では、種内で見られる内臓サイズの変異パターンを種間変異に基づいて議論されてきた高価な臓器仮説(Aiello and Wheeler 1995)の枠組みで議論した。本博士論文で得られた結果は、小進化のプロセスと大進化のプロセスは本質的に極めて類似している可能性を示唆している。しかし、本研究においては種内変異に関するデータが不足しており厳密な調査をすることはできなかった。種内変異データと種間変異データを統一的な枠組みで議論する試みは既にはじまっており、種々の理論的・手法的革新が提案されてきている（Steppan et al. 2002; Hansen and Houle 2008）。このような取り組みが本博士論文で行われた系統的種間比較法を使った研究の重要な将来像であろう。

　　まとめると、本研究では魚類の形態・生態・生活史・遺伝的多様性と系統的種間比較法を利用し、脳サイズの進化と関連する諸要因を特定した。それによって、魚類が脊椎動物の脳サイズ進化に適した研究材料であることを示すとともに、本分野における新たな研究の可能性について提案を行った。

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