

Evidence for small scale variation in the vertebrate brain: mating strategy and sex affect brain size and structure in wild brown trout (*Salmo trutta*)

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Abstract

The basis for our knowledge of brain evolution in vertebrates rests heavily on empirical evidence from comparative studies at the species level. However, little is still known about the natural levels of variation and the evolutionary causes of differences in brain size and brain structure within-species, even though selection at this level is an important initial generator of macroevolutionary patterns across species. Here, we examine how early life-history decisions and sex are related to brain size and brain structure in wild populations using the existing natural variation in mating strategies among wild brown trout (*Salmo trutta*). By comparing the brains of precocious fish that remain in the river and sexually mature at a small size with those of migratory fish that migrate to the sea and sexually mature at a much larger size, we show, for the first time in any vertebrate, strong differences in relative brain size and brain structure across mating strategies. Precocious fish have larger brain size (when controlling for body size) but migratory fish have a larger cerebellum, the structure in charge of motor coordination. Moreover, we demonstrate sex-specific differences in brain structure as female precocious fish have a larger brain than male precocious fish while males of both strategies have a larger telencephalon, the cognitive control centre, than females. The differences in brain size and structure across mating strategies and sexes thus suggest the possibility for fine scale adaptive evolution of the vertebrate brain in relation to different life histories.

Introduction

Most of the existing empirical data on brain evolution stems from comparative analyses investigating the correlates of brain size across species. Such analyses have shown significant relationships between brain size and various aspects of species' ecology which suggests brain size evolves adaptively in response to cognitive demands at the species level. For instance, variables such as diet (Hutcheon *et al.*, 2002; Gonzalez-Voyer *et al.*, 2009a), spatial ecology (Safi & Dechmann, 2005), life-history traits (Iwaniuk & Nelson, 2001), parental care patterns

(Young *et al.*, 1998; Gonzalez-Voyer *et al.*, 2009), social environment (Dunbar, 1995; Gonzalez-Voyer *et al.*, 2009a) and intensity of sexual selection (Garamszegi *et al.*, 2005; Pitnick *et al.*, 2006; but see Gonzalez-Voyer *et al.*, 2009) have all been found to co-vary with brain size across vertebrate taxa.

Although relative brain size is a robust general proxy for cognitive ability (Striedter, 2005; Pollen *et al.*, 2007), detailed analyses of the size of different brain structures provide a more exact instrument for studies aimed at disentangling fine-scale evolutionary changes in cognitive ability (Mace *et al.*, 1980; Barton & Harvey, 2000; Striedter, 2005; Gonzalez-Voyer *et al.*, 2009b). The vertebrate brain consists of several distinct structures which control specific cognitive functions (Striedter, 2005). Overlap does exist in the function of separate structures

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and one structure sometimes controls more than one function (Striedter, 2005). Still, studies correlating brain structure to potential selective pressures have provided valuable, albeit indirect, insight into the factors that have shaped the existing variation in brain structure cognitive ability among contemporary species (Striedter, 2005). One such example is found in a recent comparative study on cichlid fish, where Pollen *et al.* (2007) found support for environmental complexity and social environment affecting several brain structures in distinct ways.

In relation to the many existing comparative studies of the correlates of brain size and structure, little is known about the levels of variation in brain size and structure within species. For instance, for the most diverse vertebrate taxa: the bony fishes, the few studies that exist are limited to comparisons of wild vs. captive-raised individuals (e.g. Kihlslinger & Nevitt, 2006; Burns & Rodd, 2008), comparisons of laboratory strains (Ishikawa *et al.*, 1999) and two recent experimental studies on adaptive variation in brain size and plasticity across nine-spined stickleback (*Pungitius pungitius*) populations (Gonda *et al.*, 2009a,b). More analyses at the within-species level, ideally investigating brain variation also within wild populations, are necessary to fully understand the levels and causes of individual variation in brain size and structure in wild vertebrate populations. Speciation processes start at the level of populations (Coyne & Orr, 2004) and within-species studies both within and across populations can therefore aid in bridging the gap between microevolutionary patterns across populations and macroevolutionary patterns across species (Dobzhansky, 1937).

Brown trout (*Salmo trutta*) is a promising model for studies of within-species variation in brain size and structure since this species shows distinct mating strategies in both sexes with large differences in ecology and life histories (Elliott, 1994). Although variation exists among populations and varieties of brown trout (Elliott, 1994; Klemetsen *et al.*, 2003), in most populations (including the one under study here) adult fish spawn in freshwater where the eggs are laid in nests in the gravel on the river bottom (Elliott, 1994; Klemetsen *et al.*, 2003). Prior to sexual maturation, males either (a) assume the 'anadromous' strategy and migrate to sea to grow considerably and later return to the freshwater spawning grounds as large, dominant individuals or (b) assume the 'precocious' strategy and sexually mature earlier at smaller size (usually at less than half the total length of anadromous fish; Klemetsen *et al.*, 2003). Precocious males remain stationary in the river and take on a 'sneaker' strategy where they approach courting pairs and quickly try to fertilize eggs of egg-laying females (Elliott, 1994). Females show a similar division of strategies but precocious maturation is rarer than in males, probably due to the greater fecundity benefit in females from growing large during the sea-ward migration of anadromous individuals (Klemetsen *et al.*, 2003).

There is both a genetic and a conditional component determining which individuals end up in each strategy in Salmonids and both components are shaped by frequency dependent selection (Fleming, 1996; Klemetsen *et al.*, 2003). Interestingly, although the anadromous individuals are much larger than precocious individuals at the time of sexual maturation, the individuals with the highest level of growth during the first year tend to be the ones adopting the precocious strategy (Dellefors & Faremo, 1988). Apart from the differences in growth and spawning behaviour, there are also substantial differences in habitat, diet and social environment between strategies. Anadromous fish spend considerable time in the marine coastal environment and take on a mainly piscivorous diet whereas precocious fish tend to forage more on insects and aquatic arthropods (Keeley & Grant, 2001). Moreover, precocious fish are highly territorial in the stream whereas anadromous fish tend to form groups, at least for parts of their migrations (Elliott, 1994). Thus, there are a number of ecological, life history and behavioural differences between mating strategies in brown trout which could lead to differences in cognitive demands and ultimately to differences in relative brain size and structure.

Furthermore, sexual selection has been suggested to play an important role in brain evolution as a driver of neural dimorphism (Jacobs, 1996). Although sex-specific analyses remain scarce, a limited number of within-species comparisons and comparative analyses have demonstrated distinct patterns of brain size and structure between the sexes (Jacobs *et al.*, 1990; Bingman, 1992; Iwaniuk, 2001; Garamszegi *et al.*, 2005; Gonzalez-Voyer *et al.*, 2009a). In the brown trout, which displays 'traditional' sex-roles, males compete over choosy females (Andersson, 1994). Anadromous males not only have to find a female but also fight other males to obtain a mating while precocious sneaker males display a potentially more behaviourally complex strategy involving finding and stealthily approaching a mating pair to perfectly time the fertilization of the eggs while avoiding aggression from the large anadromous males (e.g. Olsén *et al.*, 1998). Hence, there may be substantial differences in cognitive demands across both sexes and strategies which could lead to adaptive differences in brain size and structure.

Combined evidence from previous studies, most of them analysing inter-specific patterns, have demonstrated the influence of habitat, diet, life histories, social environment, sexual selection and mating behaviours on brain size and brain structure. Here, we use the existing variation across wild brown trout mating strategies and sexes to investigate whether brain size and structure are linked to mating strategy and sex at the within-species level. We do this by comparing brain size and structure among sexually mature individuals of both mating strategies and sexes in brown trout originating from a single Swedish river in which the two strategies are

present in both sexes. This enables us to control for differences in development, and also for potential ecology-related confounding effects that could arise through comparison of individuals of different strategies from different rivers.

Methods

Data collection

Fish were collected using electro-fishing in river Jörlanda in south-western Sweden in the autumn of 2007 during one day of electro-fishing within a stretch of 900 m of the river (final sample: precocious males: $N = 11$; precocious females: $N = 12$; anadromous males: $N = 12$; anadromous females: $N = 13$). We could easily separate individuals of different mating strategies based on morphological differences (Elliott, 1994) together with notable body size differences across strategies (see Results). All caught individuals were immediately deeply anesthetized using benzocaine and measured [total length (TL), i.e. the length from the tip of the snout to the tip of the longer lobe of the caudal fin]. Following measurements, the fish were decapitated and the whole head was fixed in 4% paraformaldehyde, with a phosphate buffer, awaiting brain dissections. All fish were dissected directly after decapitation and the gonads were checked to ensure that all individuals included in the final sample were accurately sexed and had reached sexual maturity. By only including sexually mature individuals at the spawning season, we could control for the effect of neural development that can occur during the process of sexual maturation (Thompson, 1993). Brain dissections were undertaken in autumn 2008 and total brain weight was recorded for each of the brains. As all brains were dissected from the fixed skulls within a period of 2 weeks, we expect no bias in brain weights due to differences in time spent in formaldehyde.

Analysis and function of separate brain structures

For the brain structure analyses, brains were photographed through a dissection microscope (Leica MZFLIII), using a digital camera (Leica DFC 490 and FIRECAM v. 3.1 software). Separate photographs were taken for dorsal, ventral and lateral views (left side for all brains). For each image, the brain was carefully placed on a Petri dish with 0.9% agar, which was solid but would yield to brains and allow for them to be placed in such a manner to ensure that the view of the brain being photographed was horizontal and both sides were symmetrical. For paired structures [i.e. the olfactory bulbs (OB), the telencephalon (TEL) and optic tecta (OT)] we measured the structures on the left side of the brain and multiplied the volume by two (Pollen *et al.*, 2007; Gonda *et al.*, 2009a,b). Based on the pictures obtained, we followed the procedure of Pollen *et al.* (2007) to measure

length, width and height of five distinct key-structures: OB, TEL, OT, cerebellum (CER) and hypothalamus (HYP). We originally aimed at including also the pituitary gland but this structure was damaged during dissection for some of the specimens and the pituitary gland was thus disregarded from any analyses. The volume of each structure was quantified according to the ellipsoid model: $V = (L \times W \times H) \pi/6$ which has been found to provide an accurate estimate of the volume of fish brain structures (e.g. van Staaden *et al.*, 1995; Huber *et al.*, 1997; Pollen *et al.*, 2007). The OB receive olfactory signals which are then relayed directly to the TEL (Butler & Hodos, 2005; Striedter, 2005; Braithwaite, 2006). The TEL forms the cognitive centre of the vertebrate brain that processes all sensory information and also plays an important role in directing active movements as well as in learning and memory (Broglia *et al.*, 2003; Butler & Hodos, 2005; Striedter, 2005; Braithwaite, 2006). The main function of the OT, especially in fishes, is to receive visual information which is then transferred to the TEL (Butler & Hodos, 2005; Striedter, 2005; Braithwaite, 2006). The CER is the centre for motor control and coordinates muscle activity, movements and balance (Kotrschal *et al.*, 1998; Butler & Hodos, 2005; Striedter, 2005; Braithwaite, 2006). Finally, the HYP is functionally connected to the pituitary gland and controls many basic bodily functions such as reproduction and growth as well as motivation and the autonomic nervous system (Butler & Hodos, 2005; Striedter, 2005; Braithwaite, 2006). Although each structure is heterogeneous and can be further divided into even more detailed separate units (Butler & Hodos, 2005; Striedter, 2005; Braithwaite, 2006), volume estimates of these main structures generally provide a robust proxy of cognitive capabilities (Kotrschal *et al.*, 1998; Striedter, 2005).

Statistical analysis

A complicating factor in studies of the correlates of relative brain size is that brain size shows a complex allometric relationship with body size (Striedter, 2005; Deaner *et al.*, 2007; Gonzalez-Voyer *et al.*, 2009a,b). First, brain size is strongly positively correlated to body size, most likely due to increased demands on proprioception and homeostasis with increasing body size (Striedter, 2005), which is why some control for body size is usually applied in studies based on this measure of cognitive ability. Second, brain size/body size ratios increase with decreasing body size (i.e. smaller bodied species have relatively larger brains in relation to their body size than larger bodied species). Third, brain size tends to show a stronger increase with increasing body size early in development than after sexual maturation when this relationship flattens out [see Striedter (2005) for a thorough review and discussion on these three features of brain-body scaling properties]. To control for these effects of allometry, log transformation of brain size is

normally used in conjunction with inclusion of body size as a covariate following log transformation. Here, we use an ANCOVA design with total brain weight (log transformed) as the dependent variable, reproductive strategy and sex as factors and body size (log TL) as a covariate to investigate both the separate effects of reproductive strategy and sex as well as the potential interaction between these factors while controlling for body size (Quinn & Keough, 2002). For the brain structure analyses, we used total brain size as a covariate since the separate structures are usually highly correlated to total brain size (Striedter, 2005). Hence, we first used a MANCOVA including the volumes of all five brain structures (log transformed) as dependent variables, strategy and sex as factors and total brain weight as a covariate. After establishing significant effects of both strategy and sex in the multivariate analysis we then used univariate ANCOVAs for each brain structure separately to establish the effects of the factors on each of the structures (Quinn & Keough, 2002). To test for different allometric relationships across the different mating strategies and sexes, we first ran the models including all interaction terms between the factors (strategy and sex) and the covariate (body size or total brain weight) (Engqvist, 2005). As no interaction effects were significant, neither for the analysis of total brain size (strategy \times body size: $P = 0.37$; sex \times body size: $P = 0.07$; see Fig. 1 for an overview of the allometric relationships between body size and brain size for the separate strategies and sexes) nor for the analysis of brain structure (strategy \times total brain weight: $P = 0.72$; sex \times total brain weight: $P = 0.74$) these interaction terms were dropped from the final models (Engqvist, 2005). All analyses were done with STATISTICA 7.1 (StatSoft, Inc., 2005).

Results

We found a strong effect of reproductive strategy, but no effect of sex, on body size and a nonsignificant trend towards an interaction between sex and strategy (factorial ANOVA: strategy: $F_{1,44} = 216.2$, $P < 0.0001$; sex: $F_{1,44} = 0.22$, $P = 0.64$; interaction: $F_{1,44} = 3.84$, $P = 0.056$). Hence, anadromous fish were much larger than precocious fish [anadromous fish: [mean TL (mm) \pm SD] 418.4 mm \pm 70.5; precocious fish: 189.0 mm \pm 45.1]. As suggested by the interaction effect, precocious males tended (but nonsignificantly so when analysed separately) to be larger than precocious females [precocious males: (mean TL \pm SD) 203.5 mm \pm 54.6; precocious females: 175.7 mm \pm 30.8; $F_{1,21} = 2.2$, $P = 0.15$], while there was no size difference among the sexes for anadromous fish (anadromous males: 401.8 mm \pm 71.4; anadromous females: 433.8 \pm 68.7; $F_{1,23} = 1.5$, $P = 0.23$).

For the analyses of total brain size, we found a strong effect of mating strategy, but no effect of sex, and the interaction effect was statistically significant (Table 1). The covariate in the analysis, body size, was a strong

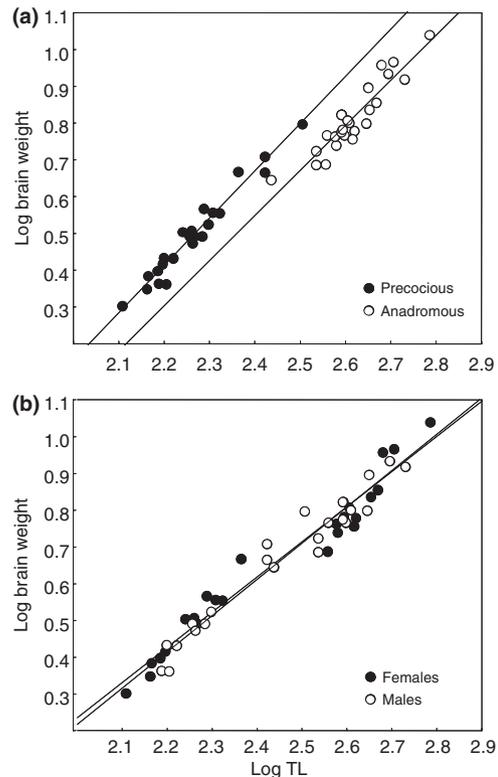


Fig. 1 Allometric relationships between body size and brain size. (a) Relationships between body size (log TL) and total brain weight for precocious ($y = -2.41 + 1.28x$) and anadromous ($y = -2.40 + 1.23x$) fish. (b) Relationships between body size (log TL) and total brain weight for females ($y = -1.68 + 0.96x$) and males ($y = -1.76 + 0.99x$).

predictor of brain size (Table 1). Thus, precocious fish had larger total brain size than anadromous fish when controlling for body size (Fig. 2a). Further analysis of the interaction showed that the effect was caused by precocious females having larger total brain size than precocious males [Fig. 2a; ANCOVA (sex as factor and body size as covariate): $F_{1,22} = 9.5$, $P = 0.006$] whereas there was no effect of sex on brain size in anadromous fish [ANCOVA (sex as factor and body size as covariate): $F_{1,22} = 0.29$, $P = 0.59$].

For the multivariate analysis of brain structure, we found a significant effect of both mating strategy and sex on the volume of the five brain structures but no interaction effect (Table 2). The covariate in the analysis, total brain weight, was a strong predictor of the volume of brain structures (Table 2). Univariate analyses (Table 3) showed that it was mainly the CER which was affected by mating strategy since anadromous fish had a larger CER as compared to precocious fish (Fig. 2b). The only brain structure significantly affected by sex (Table 3) was the TEL which was larger in males than in females (Fig. 2c). However, the olfactory bulbs and the

Table 1 Results from ANCOVA with total brain weight as dependent variable and body size as covariate. All variables were \log_{10} -transformed prior to analysis.

Effect	SS	d.f.	F	P
Body size	0.50	1	535.7	<0.0001
Strategy	0.04	1	41.1	<0.0001
Sex	0.0009	1	1.0	0.33
Strategy \times sex	0.004	1	4.2	0.046

OT showed a tendency ($P = 0.054$ and 0.09 respectively) to be larger in males than in females (Table 3). No interaction effects were detected for any of the separate brain structures (Table 3).

Discussion

Our analyses indicate surprisingly high levels of variation in both relative brain size and brain structure within a single vertebrate population. This variation was associated both with mating strategy and sex and thus suggests small-scale adaptive evolution of both relative brain size and brain structure in a vertebrate.

Precocious fish had larger brain size than anadromous fish when controlling for body size and moreover, precocious females had larger brain size than precocious males. Precocious fish defend home ranges in which they forage (Bachman, 1984), whereas anadromous fish travel in schools during the migrations and also during the time spent in the coastal waters (Elliott, 1994). Furthermore, large anadromous fish are highly aggressive towards precocious fish which are frequently attacked and injured (Elliott, 1994). These differences suggest that the potentially more complex social interactions associated with territoriality and protection of home ranges in precocious fish in combination with the need to avoid aggressive attacks from anadromous fish may have placed higher demands on cognitive ability and resulted in a larger brain (controlling for body size) in precocious fish. This view is further supported by indirect evidence from previous studies suggesting that a more complex social environment is associated with larger brain size in fishes (Bshary *et al.*, 2002; Gonzalez-Voyer *et al.*, 2009a). An alternative explanation is that there is a difference in habitat complexity between the more complex river environment of precocious fish and the simpler pelagic environment of anadromous fish. The difference in brain size between male and female precocious fish is harder to explain. Based on the complex behaviours of precocious sneaker males it is more intuitive to expect larger brains in males, thus the finding of larger brains in precocious females must be explained through a female-specific mechanism. A recent comparative study on cichlid fishes found a positive relationship between female brain size and uni-parental female care (Gonzalez-Voyer *et al.*, 2009a) which could potentially explain also our results. However, parental care behaviours in brown trout are

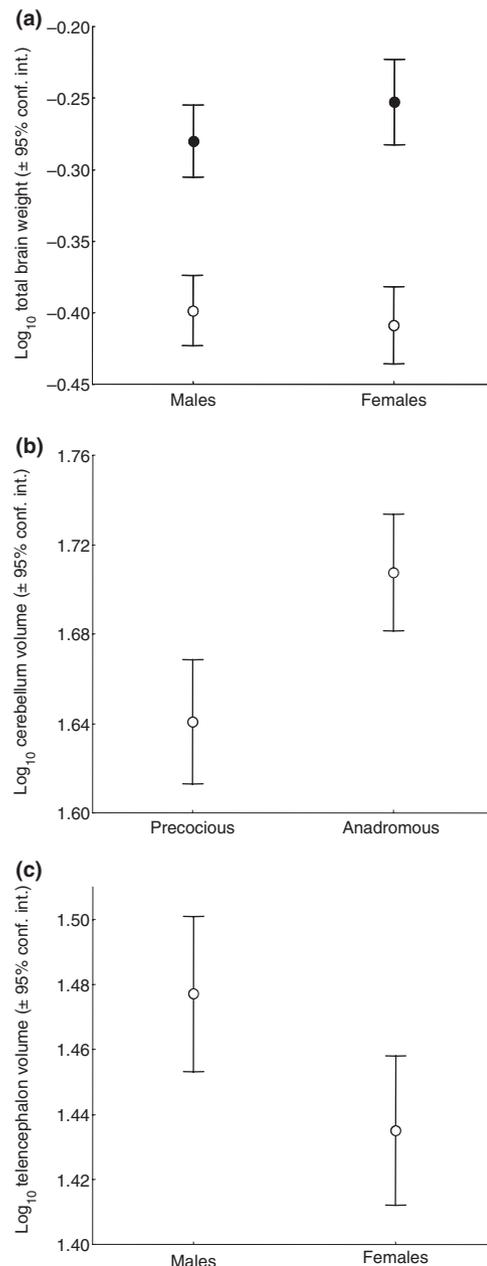


Fig. 2 Main effects for analyses of total brain size and brain structures. (a) Total brain size (LS means, corrected for covariate; result from ANCOVA with strategy and sex as factors and body size as covariate; see text and Table 1 for details) of males and females for precocious fish (filled circles) and anadromous fish (open circles). Precocious fish have larger brain size than anadromous fish; there is no effect of sex but a significant interaction effect. (b) Cerebellum volume across precocious and anadromous fish (LS means, corrected for covariate; univariate result from ANCOVA with strategy and sex as factors and total brain size as covariate; see text and Table 2 for details). (c) Telencephalon volume across males and females (LS means, corrected for covariate; univariate result from ANCOVA with strategy and sex as factors and total brain size as covariate; see text and Table 3 for details).

Table 2 Results from MANCOVA with the separate brain structure volumes (OB, TEL, OT, CER, HYP) as dependent variables and total brain weight as covariate. All variables were log₁₀-transformed prior to analysis.

Effect	Wilks λ	Effect d.f.	Error d.f.	F	P
Total brain weight	0.03	5	39	302.9	<0.0001
Strategy	0.75	5	39	2.5	0.043
Sex	0.69	5	39	3.5	0.01
Strategy \times sex	0.91	5	39	0.8	0.55

OB, olfactory bulbs; TEL, telencephalon; OT, optic tectum; CER, cerebellum; HYP, hypothalamus.

limited to females digging a shallow cavity in which the eggs are laid and thereafter covering the cavity with gravel. Thus it is unlikely that the sex difference in precocious fish can be explained through increased cognitive demands from complex female parental care behaviours, especially as we found no overall effect of sex on brain size. On the other hand, precocious females tend to lay more numerous but much smaller clutches than the larger anadromous females (Erik Petersson, personal communication) and further, small females cannot dig as effectively in courser gravel as large females (Crisp & Carling, 1989; Steen & Quinn, 1999). Thus, precocious females have to prepare numerous nests and may also have to be highly choosy regarding nest site location to avoid washout and destruction of the eggs (Crisp & Carling, 1989). Hence, we speculate that the difference in brain size among precocious males and females could be partly driven through a female parental care mechanism where cognitive demands of locating multiple suitable substrates for egg-laying select for a larger brain. More information is clearly needed on the behaviours of precocious females before we can fully understand the selective pressures leading to these results.

Interestingly, although precocious fish had larger brains than anadromous fish (when controlling for body size), the only structure which differed across mating strategies, namely the CER, was larger in anadromous than precocious fish. The CER is the control centre of movements in fishes (Finger, 1983; Braithwaite, 2006) as

well as in other vertebrates (Butler & Hodos, 2005; Striedter, 2005). Therefore, the more mobile lifestyle of the migrating anadromous fish have probably selected for increased CER size. Furthermore, a previous comparative study on cichlid fishes found a positive relationship between CER size and diet where piscivorous species, which preyed on large fish, were found to have a larger CER (Huber *et al.*, 1997). These authors suggested this resulted from the increased demands on control of movements from hunting live fishes in relation to feeding on sessile prey (Huber *et al.*, 1997). Another study on Anseriform birds found a larger CER in ducks that dive deep to feed compared to ducks that feed in shallow water (Kalisinska, 2005). Hence, the difference in foraging behaviours across different mating strategies in this brown trout population may also have contributed to the larger CER in the anadromous fish.

The effect of sex on TEL volume, together with the observed nonsignificant trends for the olfactory bulbs and optic tectum, suggest that the more complex behaviours of males during mate searching and male-male competition result in increased selection pressure on the cognitive centre of the brain. To our knowledge, this is the first demonstration of a within-species sex-difference in brain structure in fishes and this difference could result from several different mechanisms. For instance, competing and searching for mates have been suggested to be associated with high cognitive demands, particularly on learning (Jacobs *et al.*, 1990; Jacobs, 1996; Barkley & Jacobs, 2007) which is directly controlled by the TEL. Hence, those males invest most into mate searching and intra-sexual competition in brown trout certainly support sexual selection as the force behind the effect of sex on TEL volume. It would be highly interesting to perform similar comparisons in sex-role reversed species to further investigate the effect of sexual selection *per se* on brain structure.

Although our results suggest adaptive evolution of brain size and structure on a very small scale, the phenotypic plasticity of the mating strategies in brown trout (Elliott, 1994; Klemetsen *et al.*, 2003) needs consideration. For instance, there is evidence for rapid phenotypic plasticity in neural structures in Salmonids,

Table 3 Univariate results from MANCOVA with separate brain structures as dependent variables. All variables were log₁₀-transformed prior to analysis.

Structure	OB			TEL			OT			CER			HYP		
	SS	F _{1,43}	P	SS	F _{1,43}	P	SS	F _{1,43}	P	SS	F _{1,43}	P	SS	F _{1,43}	P
Total brain weight	0.72	117	***	0.81	251	***	0.49	805	***	0.81	301	***	0.64	85.5	***
Mating strategy	0.002	0.3	0.59	0.008	2.5	0.12	0.001	2.2	0.15	0.008	8.2	0.007	0.01	1.7	0.20
Sex	0.02	3.9	0.054	0.21	6.6	0.014	0.002	3.0	0.09	0.02	2.4	0.13	0.003	0.44	0.51
Mating strategy \times sex	0.006	1.0	0.32	0.006	2.0	0.17	0.0003	0.05	0.82	0.006	0.25	0.62	0.0004	0.05	0.82

***P < 0.0001.

Bold values denote statistically significant effects at $\alpha = 0.05$.

OB, olfactory bulbs; TEL, telencephalon; OT, optic tectum; CER, cerebellum; HYP, hypothalamus.

for instance in relation to habitat complexity (Kihlslinger & Nevitt, 2006). The effect we detected of sex on brain size and structure is unlikely to be driven through a mechanism of simple plasticity since all individuals were sexually mature and sex-determination tends to be genetic in Salmonids with males being the heterogametic sex (Woram *et al.*, 2003). However, as mating strategy in both sexes is partly genetically determined and partly condition-dependent in brown trout (Elliott, 1994; Klemetsen *et al.*, 2003), the differences in brain size and structure across mating strategies could partly be driven through phenotypic plasticity. A recent study investigating brain gene expression across immature males, immature females and sexually mature sneaker males of one population of Atlantic salmon, *Salmo salar*, found clear expression differences in 15% of the c. 3000 genes surveyed (Aubin-Horth *et al.*, 2005). And immature males differed from immature females in gene expression suggesting genetic sex-specific effects in brain gene expression also before sexual maturation. Experimental studies of groups of individuals of known genetic composition that are given different food ratios and then sampled both prior to actual choice of mating strategy and after sexual maturation may be a way towards fully understanding the genetic contribution behind differences in brain size and structure across mating strategies. Experimental designs which disentangle the effects of the environment from genetic effects are scarce for these types of studies but a few recent examples exist for fishes. These studies conducted on the medaka (*Oryzias latipes*: Ishikawa *et al.*, 1999), the guppy (*Poecilia reticulata*: Burns & Rodd, 2008) and the nine-spine stickleback (Gonda *et al.*, 2009a,b) have suggested adaptive evolution of brain size and structure by showing genetic population differences in neural development and also neural plasticity. However, up to now, the exact selection pressures leading to the observed variation have been difficult to elucidate and the available studies are still too scarce to allow general predictions regarding the mechanisms behind the fine-scale evolution of brain size and brain structure. Our analyses identify differences associated with life-history strategies and sex as potential mechanisms that drive the evolution of the vertebrate brain.

To conclude, we demonstrate strong effects of mating strategy and sex on brain size and brain structure within a single population of brown trout which suggest adaptive evolution of neural architecture on a remarkably small scale in vertebrates. Together with experimental designs that can elucidate the exact genetic components from environmental components behind variation in brain size and structure, we suggest these types of studies at the inter-specific level will form important future additions to comparative analyses to bridge the gap between microevolution and macroevolution regarding vertebrate brain architecture.

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