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Quantitative genetic analysis of brain size variation in sticklebacks: support for the mosaic model of brain evolution

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The mosaic model of brain evolution postulates that different brain regions are relatively free to evolve independently from each other. Such independent evolution is possible only if genetic correlations among the different brain regions are less than unity. We estimated heritabilities, evolvabilities and genetic correlations of relative size of the brain, and its different regions in the three-spined stickleback (*Gasterosteus aculeatus*). We found that heritabilities were low (average $h^2 = 0.24$), suggesting a large plastic component to brain architecture. However, evolvabilities of different brain parts were moderate, suggesting the presence of additive genetic variance to sustain a response to selection in the long term. Genetic correlations among different brain regions were low (average $r_G = 0.40$) and significantly less than unity. These results, along with those from analyses of phenotypic and genetic integration, indicate a high degree of independence between different brain regions, suggesting that responses to selection are unlikely to be severely constrained by genetic and phenotypic correlations. Hence, the results give strong support for the mosaic model of brain evolution. However, the genetic correlation between brain and body size was high ($r_G = 0.89$), suggesting a constraint for independent evolution of brain and body size in sticklebacks.

1. Introduction

The expected magnitude of evolutionary response to unit of directional selection is directly proportional to the amount of additive genetic variance in the trait under selection [1]. However, apart from additive genetic variance, genetic covariances among traits are also important. Strong genetic covariances among traits can constrain or even prevent responses to selection if the correlated changes in other traits reduce fitness [2]. Accordingly, quantitative genetics and the genetic variance–covariance matrix (**G**) occupy a central position in predicting and understanding multivariate evolution in space and time [2–6].

While studies focused on the evolution of brain size and brain architecture have a long history in evolutionary biology (e.g. [7–9]), quantitative genetic studies of brain architecture are scarce. This is understandable considering that much of the research in brain architecture has been based on comparative approaches where inferences have been drawn from patterns of interspecific variability (e.g. [9–11]). However, evolutionary studies focused on intraspecific variability have been far less common until recently (reviewed by Gonda *et al*. [12]). Accordingly, apart from studies of primates (e.g. [13–17]) and mice/rats (e.g. [18–20]), little work has been conducted on quantitative genetics of brain architecture (but see [21,22]). This is in spite of the fact such studies could aid in disentangling the two competing hypotheses of brain evolution: the mosaic model and the concerted model.

The mosaic brain evolution hypothesis postulates that different brain regions are essentially free to evolve independently of one another [23]. Conversely, the

concerted brain evolution hypothesis posits that developmental and genetic constraints make independent changes in the size of different brain regions difficult [24–26]. Neither of these hypotheses is likely to be strictly right or wrong, but the degree of non-independence among different brain regions is likely to vary, and also from one organism to another. Strong yet indirect support for the mosaic model of brain evolution has been provided by quantitative trait locus (QTL) mapping studies [27] as well as experiments demonstrating differential plasticity in different brain regions in response to environmental conditions experienced during development (e.g. [28–31]). Nevertheless, a more direct approach to address this hypothesis would require genetic data on the magnitude and patterns of genetic covariation among the size of different brain regions.

The main aim of this study was to investigate the genetic architecture of brain size variability in three-spined sticklebacks (*Gasterosteus aculeatus*), with particular emphasis on addressing one of the key distinctions between the mosaic and concerted models of brain evolution: is the evolution of different brain regions severely constrained by strong phenotypic and genetic correlations among them? To this end, we estimated the phenotypic and genetic correlations among different brain regions using quantitative genetic methods. In addition, we assessed the heritabilities and evolvabilities of the brain size and different brain regions to further evaluate their freedom to evolve or respond to environmental influences through genetic changes or phenotypic plasticity, respectively. If unconstrained by high genetic correlations, low heritabilities would enable different brain regions to respond to environmental demands through plasticity, whereas high heritabilities (and/or evolvabilities) would allow fast and independent evolution of different brain regions.

2. Material and methods

(a) Sampling and breeding

Adult three-spined sticklebacks were collected at the onset of their reproductive season (7–13 June 2011) with a seine net from the Baltic Sea in Helsinki (60°11′54″ N; 25°08′22″ E), and transported to the aquaculture facilities of the University of Helsinki. To provide optimal conditions for reproduction, the water temperature was set to 17°C and photoperiod to 24 L : 0 D cycle. The fish were fed twice a day with frozen bloodworms (*Chironomidae* sp.). Once a sufficient number of females had reached reproductive state, all the *in vitro* crosses were made within a 3 day (14–15 June 2011) time interval.

The crosses were made by over-anaesthetizing males with MS222 (tricaine methanesulfonate), dissecting their testicles and mincing them in a drop of water to make a sperm solution. The eggs from the females were gently squeezed out onto Petri dishes and the sperm solution was poured over them. The fertilized clutches were kept separately in Petri dishes filled with filtered tap water which was changed daily until hatching. The clutches were checked under a dissecting microscope (daily until hatching) and all unfertilized or dead eggs were removed. For quantitative genetic inference, we applied a paternal half-sibling design [2] in which each male ($n = 15$) was crossed with two randomly chosen females ($n = 30$ females in total). In total, 30 half- and full-sibling families were produced.

The clutches hatched about 6 days after fertilization. Twenty freshly hatched fry were used from every clutch. Four replicate pools were created by mixing five randomly selected fry from every family ($n = 150$ fry per pool). The pools were housed in 2.8 l tanks (one pool per tank) in an Allentown Zebrafish Rack

System (hereafter rack; Aquaneering Inc., San Diego, CA, USA) equipped with physical, chemical, biological and ultraviolet filters. For unknown reasons, two of the four replicates experienced mass mortality soon after they were established. On 22 July 2011, the two remaining pools were moved to large plastic tanks ($760 \times 540 \times 400$ mm) equipped with a one-way flow through water system supplying filtered tap water. To mimic summer conditions and to facilitate growth, fish were kept at 15°C water temperature and constant light during the rack-rearing period. Feeding started with live brine shrimp nauplii (*Artemia* sp.), and changed towards an *Artemia* and chopped bloodworm mix, and finally, to bloodworms. Food was provided twice a day, ad libitum. At the age of about five months (8 November), fish from the two pools were further divided into four 317 l aquaria ($1400 \times 780 \times 290$ mm). Two aquaria were subjected to an environmental enrichment treatment (electronic supplementary material, file S1), and two were kept as controls. In both treatments, the fish were maintained under a 20 L : 4 D daily regime. As these treatments had no effect on estimates of quantitative genetic parameters (electronic supplementary material, file S1), but influenced mean trait values, their effects were statistically controlled for in all analyses (see below) without further discussion.

(b) Measurements

Between 5 and 9 December (i.e. approx. one month after exposure to the treatments), all fish were over-anaesthetized with MS222. Fish were weighed with a digital balance to the nearest 0.01 g and their standard length (from tip of the mouth to end of the tail base) was measured using a digital calliper to the nearest 0.01 mm. After the measurements were recorded, brains were dissected and placed into a 4% formalin—0.1 M phosphate-buffered saline solution for fixation. At the end of the experiment, brains from 231 individuals were used for further measurements and analyses.

Total brain and brain part volumes were estimated with ellipsoid models based on three-dimensional linear measures (e.g. [29–34]). Linear measurements for the ellipsoid model were estimated from digital photographs of the dorsal, lateral and ventral sides of the brain, taken with a Sigma 105 mm macro lens mounted to a Nikon D80 digital camera. All brain photographs were taken from a standard distance and angle, with a strip of millimetre paper added for a size reference. Width, height and length of the brain and main brain regions (bulbus olfactorius, telencephalon, tectum opticum, cerebellum, hypothalamus) were measured from the photographs using TPS.DIG v. 1.37 software [35]. They were defined as the greatest distance enclosed by the given brain region as depicted in the electronic supplementary material, file S1. Total brain size was estimated in two ways: using the ellipsoid model for total brain size, and by summing the estimated sizes for all of the different brain regions. To see whether our measurements were repeatable, we repeated the full process (photographing and digital measurements) three times on 20 randomly selected brains. Repeatabilities (R; [36]) for the volume estimates were high ($0.77 < R < 0.97$; all $p < 0.001$; electronic supplementary material, file S1). This, together with the fact that the ellipsoid model estimates of brain volume were strongly correlated with the wet mass of total brain ($r = 0.94$, $t_{229} = 41.58$, $p < 0.001$) as well as with height, width and length of brain ($r \geq 0.84$, $t_{229} \geq 23.24$, $p < 0.001$) suggest that our ellipsoid model estimates of brain and brain region sizes were likely to be very good.

(c) Offspring assignment to parents and sexing

Because fish from different half-sibling families were pooled in experimental aquaria, individual offspring were assigned to their parents with the aid of microsatellite markers. After DNA

extraction (details in the electronic supplementary material, file S2), seven or more polymorphic microsatellite loci were amplified from all individuals used in the experiment and also from their parents (electronic supplementary material, file S2). Results were checked for typographical and genotyping errors as well as for null alleles with MICRO-CHECKER software [37]. Offspring assignment to parents (30 families) was conducted using a maximum-likelihood (ML) method implemented in CERVUS v. 3.0 [38]. All offspring could be assigned to their parents with high confidence (electronic supplementary material, file S2). Offspring sex was identified by amplifying a part of 3′UTR of the NADP-dependent isocitrate dehydrogenase (*Idh*) gene [39] as explained in the electronic supplementary material, file S2.

(d) Statistical and quantitative genetic analyses

An animal model approach was used to estimate levels and patterns of genetic and phenotypic variation and covariation in the data, as implemented in the *MCMCglmm* package in R [40]. The genetic basis (i.e. heritability, evolvability; see below) of the brain size and its different regions was examined using univariate animal models including sex, treatment (control or enriched), block (one of the two aquaria within a given treatment, which were nested within a treatment), and sex × treatment interaction as fixed effects. Standard length was added as a covariate to control for allometric effects. Results of the fixed effects can be found in the electronic supplementary material, file S3. Note that the use of total brain size to control for allometric effects yielded qualitatively similar results and conclusions. An 'animal' term linked to the pedigree (estimating additive genetic variance), and a 'dam' term accounting for possible maternal effects were included into the models as random effects. An inverse Wishart prior ($V = 1$, $\nu = 0.002$) was used for the variance component estimation, and the models were run for 5 000 000 iterations discarding the first 2 500 000 runs as burn-in, and sampling every 500th iteration. As a result, 5000 samples from the posterior distribution were obtained. The models were run both with and without pedigree information, and comparisons of deviance information criterion (DIC) revealed that models with the pedigree information were always better than those without (electronic supplementary material, table S1).

To examine the multivariate relationship (i.e. phenotypic and genetic correlations) between different brain regions, a series of bivariate models were run, first on the phenotypic level and then at the genetic level. Fixed effect structure was as for univariate models, and an inverse/Wishart prior (V = phenotypic variance/4, $\nu = 2$) was also used. Bivariate phenotypic models were run for 1 000 000 iterations, the first half of which were discarded and every 500th iteration was sampled, yielding 1000 posterior samples. For the genetic models, the number of iterations was increased to 15 000 000, discarding the first 5 000 000, yielding 20 000 posterior estimates. When estimating the genetic correlation between total brain size and standard length, 10 000 000 iterations were used. Posterior distributions and autocorrelations of all models were visually inspected to ensure good mixing of the chains and low (<0.1) autocorrelation of estimates.

To further investigate patterns of genetic and phenotypic integration in brain, principal component analysis (PCA) was performed on genetic (**G**) and phenotypic (**P**) correlation matrices using prcomp function implemented in the *stats* package in R [41]. Similarity of **G**- and **P**-matrices were compared with a Mantel's test with 10 000 permutations using the *vegan* package in R [42]. Partial genetic and phenotypic correlations were used to evaluate correlations remaining between two brain regions with effects of all other brain regions partialled out. They were calculated directly from genetic and phenotypic correlation matrices using the *corpcor* package in R [43].

Brain–body size allometry was evaluated to get further insights on possible constraints for brain size evolution (cf. [7]). The allometric coefficient (b) of brain–body size relationship was estimated from a linear mixed effect model, treating brain size as a response variable, standard length as a covariate, and sex, treatment and sex × treatment as fixed factors. Sire and dam (nested within sire) effects were included as random effects. To probe whether the observed level of allometry is probably owing to: (i) brain size evolving as a response to selection acting on body size, or (ii) allometry evolving as a response to selection acting on brain size, we used the following two equations from Lande [7]:

$$\alpha_1 = \gamma \frac{\sqrt{h^2_{\text{brain}}} \times \text{CV}_{\text{Pbrain}}}{\sqrt{h^2_{\text{body}}} \times \text{CV}_{\text{Pbody}}} \tag{2.1}$$

and

$$\alpha_2 = \frac{1}{\gamma} \frac{\sqrt{h^2_{\text{brain}}} \times \text{CV}_{\text{Pbrain}}}{\sqrt{h^2_{\text{body}}} \times \text{CV}_{\text{Pbody}}}. \tag{2.2}$$

Here, α_1 and α_2 refer to allometric slopes in situations where selection is acting only on body size (α_1) or on brain size (α_2), γ is the genetic correlation between brain and body size, h^2 is heritability and CV_P is the coefficient of phenotypic variation, which is approximately equal to the standard deviation of ln transformed trait [7].

All estimated parameters (h^2, CVs, r's) are reported as posterior modes with the 95% highest posterior density intervals (95% HPDI) unless otherwise noted. In all analyses, all traits were $\log_{10}$ transformed prior the analyses. However, coefficients of additive genetic (CV_A), phenotypic (CV_P) variation and evolvability (I_A) were obtained from models without transformations (cf. [44]). CV_A's were estimated by dividing $\sqrt{V_A}$ with the trait mean, whereas I_A was estimated by dividing V_A with squared trait mean [45].

Finally, we note that all quantitative genetic parameters (i.e. heritabilities, evolvabilities, allometries and genetic correlations) were also estimated using alternative proxies of brain size (*viz.* brain mass, height, width and depth) and brain regions (*viz.* height, width and depth) to assess the robustness of our inference. Since the usage of these alternative proxies returned results and conclusions similar to those obtained using ellipsoid model estimates, only the latter are reported.

3. Results

(a) Heritabilities and evolvabilities

Heritability estimates of brain size and the size of different brain regions were relatively low (mean $h^2 = 0.24 \pm 0.08$ (s.d.)) and roughly similar across different traits (table 1). Maternal effect influences on all brain traits were significant, and generally lower than the additive genetic effects, with the exception of brain size for which the maternal effects coefficient ($V_M/V_P = 0.40$) exceeded its heritability (table 1). Phenotypic coefficients of variation (CV_P) averaged at 12.17% (± 5.98 (s.d.); electronic supplementary material, table S1) and were higher than their genetic counterparts (mean $\text{CV}_A = 5.50 \pm 2.43\%$ (s.d.); table 1). Evolvabilities for brain size and brain regions averaged at 0.30% ($\pm\ 0.21$ (s.d.); table 1). Heritability of body size ($h^2 = 0.34$; HPDI: 0.15–0.55) was of similar magnitude to that of the size of different brain regions (table 1).

(b) Phenotypic, genetic and environmental correlations

Both phenotypic (mean $r_P = 0.33 \pm 0.16$ (s.d.)) and genetic correlations (mean $r_G = 0.40 \pm 0.27$ (s.d.)) among different

Table 1. Variance component, narrow sense heritability (h^2) and evolvability (I_A) estimates brain size and size of different brain regions. (V_A, additive genetic variance; V_M, maternal effect variance; V_R, residual variance, V_P, phenotypic variance, CV_A, coefficient of additive genetic variance. The estimates were obtained using $\log_{10}$ transformed values, except for CV_A and I_A. HPDI, highest posterior density interval. All variance components multiplied by 100.)

trait	V_A (95% HPDI)	V_M (95% HPDI)	V_R (95% HPDI)	V_P (95% HPDI)	h^2 (95% HPDI)	$CV_A\%$ (95% HPDI)	$I_A\%$ (95% HPDI)
bulbus olfactorius	0.20 (0.03–0.61)	0.09 (0.02–0.27)	0.59 (0.30–0.75)	0.89 (0.72–1.17)	0.14 (0.03–0.58)	7.98 (4.33–17.45)	0.63 (0.13–2.92)
cerebellum	0.08 (0.03–0.28)	0.05 (0.02–0.14)	0.18 (0.07–0.23)	0.34 (0.27–0.47)	0.33 (0.09–0.68)	6.69 (3.34–12.35)	0.38 (0.08–1.44)
hypothalamus	0.04 (0.02–0.15)	0.03 (0.01–0.08)	0.18 (0.12–0.23)	0.28 (0.22–0.35)	0.18 (0.07–0.47)	4.02 (2.27–8.04)	0.16 (0.04–0.62)
tectum opticum	0.05 (0.02–0.12)	0.04 (0.02–0.09)	0.06 (0.03–0.10)	0.17 (0.14–0.23)	0.31 (0.13–0.62)	5.51 (2.86–8.95)	0.30 (0.06–0.76)
telencephalon	0.09 (0.02–0.28)	0.05 (0.02–0.15)	0.27 (0.16–0.36)	0.46 (0.36–0.59)	0.17 (0.06–0.54)	7.36 (3.33–12.84)	0.31 (0.07–1.55)
brain size	0.01 (0.01–0.01)	0.01 (0.01–0.02)	0.01 (0.01–0.01)	0.03 (0.02–0.04)	0.31 (0.20–0.43)	1.47 (0.79–2.50)	0.02 (0.01–0.06)
brain size (sum of the parts)	0.05 (0.02–0.11)	0.03 (0.01–0.08)	0.06 (0.03–0.09)	0.15 (0.12–0.21)	0.32 (0.13–0.60)	5.75 (2.72–8.29)	0.20 (0.06–0.65)
body size	0.03 (0.01–0.07)	0.03 (0.01–0.06)	0.05 (0.02–0.06)	0.11 (0.09–0.15)	0.34 (0.15–0.55)	3.37 (1.47–6.22)	0.07 (0.01–0.36)

brain regions were relatively low (table 2), but highly correlated with each other (Mantel test; $r = 0.90$, $p < 0.01$). The largest genetic correlation ($r_G = 0.78$; HPDI: 0.06–0.92) was observed between the telencephalon and optic tectum, but as in the case of the other correlations, this correlation was also significantly less than unity (table 2).

The relatively low degree of both phenotypic and genetic integration in the stickleback brain was also obvious from the PCA results: although the eigenvalues were low, the second to fifth eigenvectors tended to load heavily on a single variable in both **P** and **G** matrices, suggesting that they effectively described variation in one brain region (electronic supplementary material, table S2). Likewise, partial correlations among different matrix elements were mostly low both for **P** and **G** (table 3), suggesting a high degree of independence among different brain regions. Notably, the highest phenotypic and genetic partial correlations occurred between the telencephalon and optic tectum, and between the optic tectum and cerebellum (table 3). These are also the brain regions showing the highest genetic correlations among each other (table 2).

(c) Brain–body size allometry

In spite of the strong positive genetic correlation between brain and body size ($r_G = 0.89$; HPDI = 0.12–0.96), the (hypo)allometric relationship between brain and body size had a relatively low allometric coefficient ($b = 0.33 \pm 0.03$ (s.e.)). Solving equation (2.1) yielded an estimate of allometric slope $\alpha_1 = 0.27$ (HPDI: 0.04–0.51), whereas the corresponding estimate from equation (2.2) was $\alpha_2 = 1.04$ (HPDI: 0.22–1.94). Hence, the allometric coefficient obtained assuming selection acting only on body size (α_1) is more similar to the observed brain–body size allometry (b) than that obtained assuming selection mainly on brain size (i.e. α_2).

4. Discussion

The most salient finding of this study was the relatively weak phenotypic and genetic integration of the three-spined stickleback brain. Estimated phenotypic and genetic correlations among different brain regions were relatively low, and the genetic correlations were significantly less than unity. These findings give support for the mosaic model of brain evolution, according to which natural selection can change one brain area without being constrained by genetic correlations with other areas [23–26,46]. The relatively low genetic correlations were accompanied by low heritabilities of different brain regions. This finding lends further support for the mosaic model of brain evolution: as there is only a small genetic component to the size of different brain regions, they have the freedom to respond to environmental demands through plasticity.

The central tenet of the 'strict' concerted model of brain evolution is that different brain regions are not free to evolve independently from each other. Although not often expressed in quantitative genetic terms (but see [12]), this translates to the expectation of high genetic correlations among brain regions. We found that the genetic correlations were on average moderate at best, and all significantly less than unity. Hence, although some of the individual correlations were high, independent selection responses in different brain regions should still be possible. These results are in agreement with the findings of a QTL study showing that different major loci are involved in determining the size of different brain regions in mice [27].

Table 2. Genetic (r_G; below diagonal) and phenotypic (r_P; above diagonal) correlations between different brain regions. (Statistically significant correlations are in italics and values inside the brackets indicate highest posterior density interval (HPDI).)

trait	bulbus olfactorius	cerebellum	hypothalamus	tectum opticum	telencephalon
bulbus olfactorius		*0.21* (0.08 – 0.32)	*0.24* (0.14 – 0.38)	*0.18* (0.03 – 0.28)	*0.18* (0.05 – 0.30)
cerebellum	0.29 (−0.59 to 0.75)		*0.24* (0.10 – 0.36)	*0.54* (0.44 – 0.63)	*0.47* (0.34 – 0.55)
hypothalamus	0.06 (−0.60 to 0.72)	0.37 (−0.53 to 0.77)		*0.28* (0.13 – 0.37)	*0.32* (0.21 – 0.45)
tectum opticum	0.22 (−0.56 to 0.74)	0.71 (−0.06 to 0.89)	0.46 (−0.45 to 0.78)		*0.64* (0.56 – 0.72)
telencephalon	0.03 (−0.67 to 0.69)	0.72 (−0.11 to 0.91)	0.35 (−0.44 to 0.81)	*0.78* (0.06 – 0.92)	

Table 3. Partial genetic (below diagonal) and phenotypic (above diagonal) correlations between brain regions.

trait	bulbus olfactorius	cerebellum	hypothalamus	tectum opticum	telencephalon
bulbus olfactorius		0.11	0.18	0.03	0.03
cerebellum	0.32		0.06	0.34	0.17
hypothalamus	−0.08	0.12		0.06	0.17
tectum opticum	0.22	0.19	0.29		0.50
telencephalon	−0.34	0.45	−0.08	0.58	

The low degree of integration in the stickleback brain was also evident from the phenotypic data, which is the level at which selection operates. After controlling for the influence of other brain regions on variation in a given bivariate correlation, little correlation remained, suggesting that the shared genetic influence on brain traits is quite small. Likewise, spectral decomposition of the phenotypic and genetic correlation matrices among different brain regions using PCA revealed that after extracting the first eigenvector, the variation captured by the subsequent eigenvectors in both phenotypic and genetic matrices were typically attributable to single brain regions. All this suggests a low level of integration in the stickleback brain and supports the mosaic model of brain evolution.

Brain size typically shows an (hypo)allometric relationship with body size, and hence, selection on one trait is expected to lead to correlated responses in the other trait. For instance, it has been suggested that in closely related mammalian taxa, brain size differentiation would have occurred mainly as a correlated response to directional selection acting on body size [9]. By contrast, Gonzalez-Voyer *et al.* [47] suggested that brain and body size have been free to evolve relatively independently in African cichlid taxa. However, such comparative analyses are based on a number of assumptions. One such assumption is that the observed phenotypic patterns in allometries reflect underlying genetic allometries [7,20,48]. Our results show that there is indeed a strong genetic correlation between brain and body size, suggesting a constraint for independent evolution of brain and body size in sticklebacks. Comparisons of observed and expected allometric slopes under different evolutionary scenarios supported this view: the observed allometry was more compatible with the model assuming that brain size

has evolved as a correlated response to selection on body size, rather than as a response to selection on brain size.

The observed low heritability of the total brain size and its different parts is in stark contrast with the estimates from human studies, which typically show very high ($h^2 \approx 0.66–0.97$) heritabilities of various anatomical brain features including size and/or volume of the total brain and its different parts (e.g. [17,49]). Relatively high heritability estimates of total brain size have also been reported from other primate species [50,51], rodents [18,19] and birds [21]. However, these are all taxa with determinate growth, whereas fishes exhibit indeterminate growth and neurogenesis that continues throughout life in all parts of the brain [52,53]. Hence, the relative contribution of environmental influences on fish brain architecture may exceed that seen in vertebrate taxa with determinate growth. However, the data on this effect are still scant, and to the best of our knowledge, there is only one earlier study that has focused on heritability of brain size in fishes [22]. Nevertheless, the low heritability of the size of the brain and its different parts in sticklebacks is compatible with a large body of research showing a high degree of phenotypic plasticity in these traits in various fish species [28–31,34,54,55], including the three-spined stickleback [56]. Also noteworthy in this context are the significant and sometimes relatively large (in comparison to heritabilities) maternal effect influences on the size of different brain regions found in our study. Although the study design does not allow partitioning of maternal effect influences into their genetic and environmental components, the fact that a large fraction of the variability in brain traits is attributable to environmental and maternal effect influences underlines the importance of factors other than additive genetic effects as determinants of phenotypic variance in fish brain.

Although heritability can predict short-term evolutionary responses to selection [1,2], low heritabilities, such as those observed in this study, do not necessarily implicate low evolvability: large environmental and non-additive genetic contributions to phenotypic variance can hide substantial additive genetic variance in a trait [45,57]. The observed coefficients of additive genetic variance for different brain regions were moderately high (average $CV_A = 6.31\%$) and similar to those typically observed for fitness-related life-history traits [56]. Similarly, the evolvabilities (I_A) of different brain regions—indicative of the expected change (%) in the trait mean if the trait was subject to unit selection [45]—were moderate (average $I_A = 0.35\%$) and similar to those usually observed in life-history and physiological traits [45]. However, the CV_A and I_A for total brain size were low ($CV_A = 1.47\%$; $I_A = 0.02\%$). For instance, the average CV_A in human brain size as estimated across 28 different studies is 7.8% [58], and evolvabilities as low as those estimated in this study are typically encountered only in studies focused on the genetics of developmental instability [45]. The low evolvability estimates for brain size could be indicative of it having been subject to history of strong stabilizing selection (cf. [58]). However, in the view that brain size is a high-dimensional composite trait which should accumulate genetic variance through all genes influencing its different parts, this explanation seems unlikely. Instead, we suspect that the low evolvability estimates (i.e. CV_A and I_A) in this study are caused by the ellipsoid model underestimating variability in the total brain size in this species. This possibility is supported by the facts that: (i) if brain size is estimated as a sum of the different brain regions, CV_A and I_A estimates rebounded to the levels observed for different brain regions, and (ii) the evolvability estimates for brain mass ($CV_A = 5.67\%$, $I_A = 3.2\%$) are very similar to the sum-of-parts estimates. Hence, we conclude that evolvabilities of the size of the brain and its different regions appear to be similar to those observed in human studies.

The ellipsoid model [33] we used to estimate the size of the different brain regions is likely to have lower resolution than more sophisticated methods such as magnetic resonance imaging [58] and histology (e.g. [27]) that are increasingly used to characterize variability in brain structures. However, the ellipsoid model is widely used in evolutionary studies of brain size variability and has been shown to yield reasonable estimates of the size of different brain regions [33]. Furthermore, we have no reason to believe that estimates from the ellipsoid model would bias the estimates of quantitative genetic parameters, especially since: (i) the repeatability estimates for all of the brain regions were very high (electronic supplementary material, file S1), and (ii) because ellipsoid model (sum-of-parts) and mass estimates of brain size returned similar variance (viz. h^2, CV_A, I_A) and covariance (viz. r_G, r_P) estimates.

Furthermore, by inference, if the ellipsoid model estimates for different brain regions had been poor, we would not have expected the sum-of-parts estimates for total brain size correlate strongly ($r = 0.94$) with the mass based estimate of total brain size. Another limitation of our study is the relatively low power to estimate higher order quantitative genetic parameters such as genetic correlations. However, although the credible intervals surrounding our posterior modes were large, the estimates were still accurate enough to show that they did not encompass unity. Likewise, they are not expected to be biased [59]. Finally, we denote that our inference is based on estimates obtained from a single population in particular environmental conditions and hence the results might not apply to other populations and environments [2,48]. However, given that we used F_1-offspring from wild collected parents from an outbred population, the estimates should not at least be biased by inbreeding effects.

In conclusion, the results give strong support for the mosaic model of brain evolution, showing that genetic correlations among different brain regions are relatively low and significantly less than unity. The low heritabilities of the size of the brain and its different parts suggest an important role of phenotypic plasticity in shaping the size of different brain regions—a suggestion also supported by data accumulated from empirical studies of neural plasticity in fishes. In spite of the relatively high degree of genetic and phenotypic independence among the different brain regions, the high genetic correlation between brain and body size suggests that allometry may constrain independent evolution of stickleback brain and body size.

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