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Brain size variation in extremophile fish: local adaptation versus phenotypic plasticity

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Abstract

The brain is a plastic organ, and so intraspecific studies that compare results obtained from wild individuals with those from common-garden experiments are crucial for studies aiming to understand brain evolution. We compared volumes of brain regions between reproductively isolated populations of a neotropical fish, *Poecilia mexicana*, that has locally adapted to perpetual darkness (Cueva Luna Azufre), toxic hydrogen sulphide in a surface stream (El Azufre) or a combination of both stressors (Cueva del Azufre). Wild fish showed habitat-dependent differences: enlarged telencephalic lobes and reduced optic tecta were found in fish living in darkness and sulphidic waters, in darkness without hydrogen sulphide or exposed to light and sulphide; fish from the sulphidic cave additionally showed enlarged cerebella. Comparison with common-garden reared fish detected a general decrease in brain size throughout populations in the lab, and little of the brain size divergence between lab-reared ecotypes that was seen in wild-caught fish. The pronounced differences in brain region volumes between ecotypes in the wild might be interpreted within the framework of mosaic evolution; however, the outcomes of common-garden experiments indicate a high amount of phenotypic plasticity. Our study thus highlights the importance of combining the investigation of brain size in wild populations with common-garden experiments for answering questions of brain evolution.

Introduction

The brain is the most important organ of perception (Jacyna, 2009) but also an energetically costly tissue (e.g. Isler & van Schaik, 2006). According to the 'expensive tissue hypothesis', the benefits of increased brain size must therefore outweigh the associated costs (e.g. Aiello & Wheele, 1995; Atwell & Laughlin, 2001; Kotrschal *et al*., 2013). Two hypotheses exist that try to explain the wide range of differences in brain size observed between vertebrate species. (1) The mosaic evolution hypothesis assumes trait modularity, so that parts of a functional system can change independently, allowing for rapid adaptive trait divergence (Liem, 1978; Hulsey, Garcia de León & Rodiles-Hernández, 2006). Accordingly, natural selection can influence one brain area independently from other areas leading to mosaic evolution (Barton & Harvey, 2000; de Winter & Oxnard, 2001; Hager *et al*., 2012). Because brain tissue is metabolically costly (Isler & van Schaik, 2006), selection under this scenario could specifically target particular brain regions instead of affecting the size of the whole brain, thus keeping surplus energy expenses at a minimum (Striedter, 2005; Gonzalez-Voyer, Winberg & Kolm, 2009). (2) The concerted evolution hypothesis assumes constraints to brain region evolution because of developmental interdependencies (Finlay & Darlington, 1995; Finlay, Darlington & Nicastro, 2001; Yopak *et al*., 2010). Hence, proportions of brain regions would remain unchanged, while total brain size is scaled up or down in response to specific selection on certain areas.

In contrast to amniotes (especially mammals), fishes show considerable neurogenesis during adulthood, that is, lifelong growth of the brain (Zupanc, 2001; Charvet, Striedter & Finlay, 2011). Moreover, the brain is known to be a highly

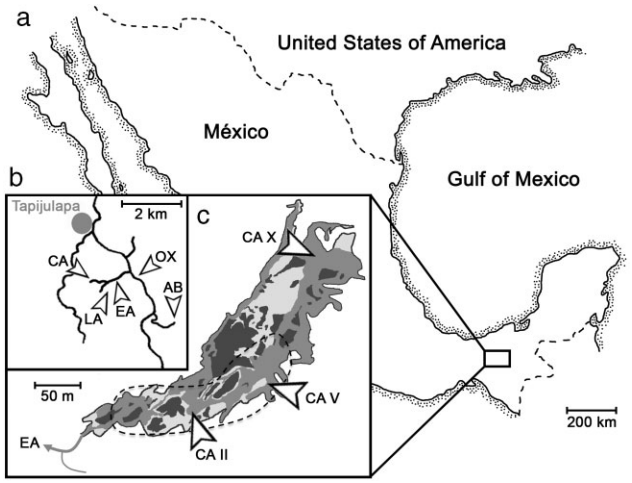

Figure 1 (a) Location of the study area near Tapijulapa, in Tabasco, Mexico. (b) The studied sites Arroyo Bonita (AB, non-sulphidic surface habitat), El Azufre (EA, sulphidic surface habitat), Cueva del Azufre (CA, sulphidic cave) and Cueva Luna Azufre (LA, non-sulphidic cave) are in close spatial proximity to each other. (c) Map of the Cueva del Azufre illustrating the different cave chambers II, V and X (CA-II, -V, -X).

plastic organ that can adapt rapidly to its environment (Nottebohm, 1981; Hofmann 2003; Gonda, Herczeg & Merilä, 2009*b*, 2011; Kotrschal *et al.*, 2012*b*). In consequence, the question arises of whether the observed differences in brain size between species or populations are mainly phenotypically plastic or genetically fixed. A number of studies on brain size divergence in fishes were conducted only on wild specimens (e.g. Kotrschal, van Staaden & Huber, 1998; Pollen *et al.*, 2007; Gonzalez-Voyer, Kolm & Iwaniuk, 2010) and seem to provide good evidence for the mosaic evolution hypothesis. Studies on sticklebacks and guppies, however, indicated a high degree of phenotypic plasticity in brain size (Gonda *et al.*, 2011, 2012; see also references cited in Burns & Rodd, 2008; Burns, Saravanan & Rodd, 2009; Gonda, Herczeg & Merilä, 2013). A recent key paper on brain evolution (Gonda *et al.*, 2013), therefore, emphasizes that before testing any evolutionary questions regarding brain size and architecture, more intraspecific studies are needed that combine investigations of wild-caught fish with common-garden experiments in order to estimate the amount of genetically based differences. The few intraspecific studies focusing on this issue (Gonda, Herczeg & Merilä, 2009*a*; Crispo & Chapman, 2010; Gonda *et al.*, 2011) demonstrated the need for such study designs as they found brain size variation among wild populations to be highly phenotypically plastic.

Populations of the Atlantic molly (*Poecilia mexicana*) inhabiting surface and cave habitats in south-eastern Mexico (Cueva del Azufre system, Tabasco; Fig. 1a and b) provide an ideal model system to disentangle the underlying mechanism for brain size divergence (i.e. heritability vs. phenotypic plasticity). Several populations in this system show strong patterns of local adaptation (Plath *et al.*, 2007; Tobler *et al.*, 2008*a*; Riesch, Plath & Schlupp, 2010) and emergence of reproductive isolation (Tobler *et al.*, 2009; Plath *et al.*, 2010, 2013). They have adapted to two co-occurring sources of selection: the presence or absence of light between surface and cave habitats and presence or absence of toxic hydrogen sulphide (H_2S) between habitats with different water sources (Tobler *et al.*, 2008*a,b*). The two factors occur in all combinations, so besides regular surface streams inhabited by ancestral *P. mexicana*, the same species inhabits a sulphidic surface creek (El Azufre, hereafter EA), a non-sulphidic cave (Cueva Luna Azufre, hereafter LA), and different chambers of a sulphidic cave (Cueva del Azufre, hereafter CA; Fig. 1c).

Our study system is particularly well suited for testing whether intraspecific brain size divergence is a result of local adaptation driven by natural selection or whether brain variation is due to adaptive phenotypic plasticity enabling fish to cope with certain (extreme) environmental conditions in their habitats. While many studies necessarily rely on comparisons among distantly related taxa with vastly different evolutionary histories, our system is evolutionarily young, with speciation in its incipient stages (Tobler & Plath, 2011). This allows the investigation of brain evolution among populations of the same species within a restricted geographic area, effectively minimizing variability of brain morphology as a result of different phylogenetic backgrounds. Previous studies in this system have demonstrated that divergent natural selection because of permanent darkness and toxic H_2S are the key drivers of evolutionary diversification in a number of traits (e.g. Tobler *et al.*, 2008*a*, 2011; Riesch *et al.*, 2010; Riesch, Plath & Schlupp, 2011; Tobler & Plath, 2011), and there are clear *a priori* predictions for adaptive brain differentiation in response to these two natural stressors.

CA fish were shown to have elaborated non-visual sensory systems, including a higher number of taste buds and an elaborated mechanosensory lateral line system (Parzefall, 1970, 2001), as well as the ability to perform female mate choice in darkness (Tobler, Schlupp & Plath, 2008*c*). CA fish further exhibit a morphocline in various morphological and sensory features (Plath *et al.*, 2007; Fontanier & Tobler, 2009); for example, eyes decrease gradually in size from front cave chambers that receive some light to the permanently dark inner chambers (Plath *et al.*, 2007). Accordingly, the optic tectum – being primarily responsible for the processing of visual input while also receiving multisensory input (Butler & Hodos, 2005) – should be reduced in cave populations (LA and CA) and could display the same cline-like reduction pattern in CA fish as reported for eye size and opsin gene expression (Tobler *et al.*, 2010). The cave environment should further promote changes in brain size because of the increasing importance of non-visual (chemical and mechano-sensory) senses (e.g. Trajano, 1994).

H_2S and the correlated hypoxia seem to lead to reduced brain size (Chapman & Hulen, 2001; Crispo & Chapman, 2010), which is in accordance with the idea that metabolic maintenance costs and high oxygen consumption of brain tissue drive brain size evolution (Isler & van Schaik, 2006). Swamp-dwelling forms of two mormyrid species

Table 1 Overview of the studied sites, abbreviations, sample sizes (*n*), habitat types and presence or absence of the toxicant hydrogen sulphide (H_2S)

Site	Abbreviation	*n* (lab)	*n* (wild)	Habitat	H_2S
Arroyo Bonita	AB	–	11/4	Surface	No
Río Oxolotán	OX	5	–	Surface	No
El Azufre	EA	4	13/4	Surface	Yes
Cueva del Azufre	CA; chamber II	3	11/7	Cave	Yes
	CA, chamber V	5	9/11	Cave	Yes
	CA, chamber X	3	11/8	Cave	Yes
Cueva Luna Azufre	LA	5	4/2	Cave	No

Note that sample sizes of lab-reared fish refer to females only, whereas sample sizes of wild animals include both sexes (females/males).

(*Gnathonemus victoriae*, *Petrocephalus catostoma*) that have to cope with permanent hypoxia showed significantly smaller brains that their counterparts thriving in well-oxygenated habitats (Chapman & Hulen, 2001). Energy availability is constrained because of foraging trade-offs – given that poeciliids in sulphidic waters spend considerable time engaged in aquatic surface respiration but must venture to the bottom for feeding (Tobler *et al.*, 2009*a*) – and the continuous detoxification of H_2S (Bagarinao, 1992). Thus, we predicted reduced overall brain size in *P. mexicana* from sulphidic habitats (EA and CA).

In our present study, we compared dimensions (volume estimates) of five brain areas (olfactory bulbs, telencephalic lobes, optic tectum, corpus cerebelli, hereafter called cerebellum and hypothalamus), the size of the whole brain, olfactory nerve and eye size of *P. mexicana* from regular surface habitats and from sulphidic and/or subterranean habitats including a comparison of fish from different CA cave chambers. In line with the starkly opposing environmental conditions outlined above, we asked the following questions: (1) do ecotypes show variation in total brain size and/or brain regions?; (2) if intraspecific differences occur, is this variation caused by local adaptation or phenotypic plasticity? Our study is the first to test heritability vs. plasticity of brain variation in a system with incipient ecological speciation. Therefore, divergent ecotypes of *P. mexicana* are an excellent model to provide further insights into the proximate and ultimate causes for brain size divergence in fishes.

Materials and methods

Study system and sample origin

Poecilia mexicana were collected from six sites in July 2013 (Fig. 1; for sample sizes and further details, see Table 1), namely the Arroyo Bonita (surface, non-sulphidic; AB), the El Azufre (surface, sulphidic; EA), chambers II, V and X of the Cueva del Azufre (cave, sulphidic; CA-II, CA-V and CA-X) and the Cueva Luna Azufre (cave, non-sulphidic; LA).

We included fish from a population level common-garden rearing experiment. Laboratory stocks of fish were available from all relevant populations; the stock for AB, however, was a mixed population consisting of fish originating from several non-sulphidic surface habitats of the Río Oxolotán (OX) system, including AB. All stocks were founded by dozens of individuals each in January 2006 and maintained as randomly outbred populations in 1000-L flow-through tanks in a temperature-controlled greenhouse at the Aquatic Research Facility of the University of Oklahoma (one tank per population). All stocks were exposed to identical environmental conditions, that is, ambient light conditions and no H_2S or predators. Algae, detritus and invertebrates were present in the stock tanks, and the diet was supplemented with commercial flake food twice a week. Random samples of females from these stocks were collected in June 2009 (Table 1). At this point, the stocks were established in the laboratory for multiple (≥4th) generations. As stocks were of limited size, we used only female laboratory animals.

Brain preparation and measurements of brain and eye size

Fish were anesthetized and euthanized with an overdose of tricaine methanesulfonate (MS-222, Sandoz, Rotkreuz, Switzerland) and fixed in 5% phosphate buffered glutaraldehyde. Standard length and eye size (maximum diameter) were measured for each individual with a sliding calliper to the nearest 0.05 mm. Heads were detached from the body and soaked in deionized water for one hour; brains were then dissected and stored in 5% phosphate buffered glutaraldehyde until performing measurements.

Dissected brains were placed on a 0.1% agarose gel and photographed through a stereo-microscope using a Nikon DS-Fi1 camera in lateral, ventral and dorsal projections. Length, width and height of the total brain, telencephalic lobes, optic tectum, cerebellum, hypothalamus, olfactory bulb and diameter of the olfactory nerve were measured using the software NIS-Elements BR 3.2 (Nikon Corp. Tokyo, Japan; Fig. 2). Brain measurements followed the protocol of Pollen *et al.* (2007), with the exception of total brain length, which was measured from the telencephalic lobes to the cerebellum excluding the medulla oblongata as it was cut imprecisely. Regarding the width of the olfactory bulbs, telencephalic lobes, optic tectum and hypothalamus, both hemispheres were measured and mean values calculated for further statistical analyses. At the end of all linear measurements, brains were weighed to the closest 0.01 g.

Statistical analyses

Brain volumes

To obtain estimates of the overall size of the total brain and each brain region (olfactory bulbs, telencephalic lobes, optic tectum, cerebellum, hypothalamus), we calculated the volumes according to the ellipsoid volume formula (e.g. Pollen *et al.*, 2007) using length, width and height measurements of the brain or the respective brain region:

$$\text{Volume} = (\text{length} \times \text{width} \times \text{height}) \times \pi/6.$$

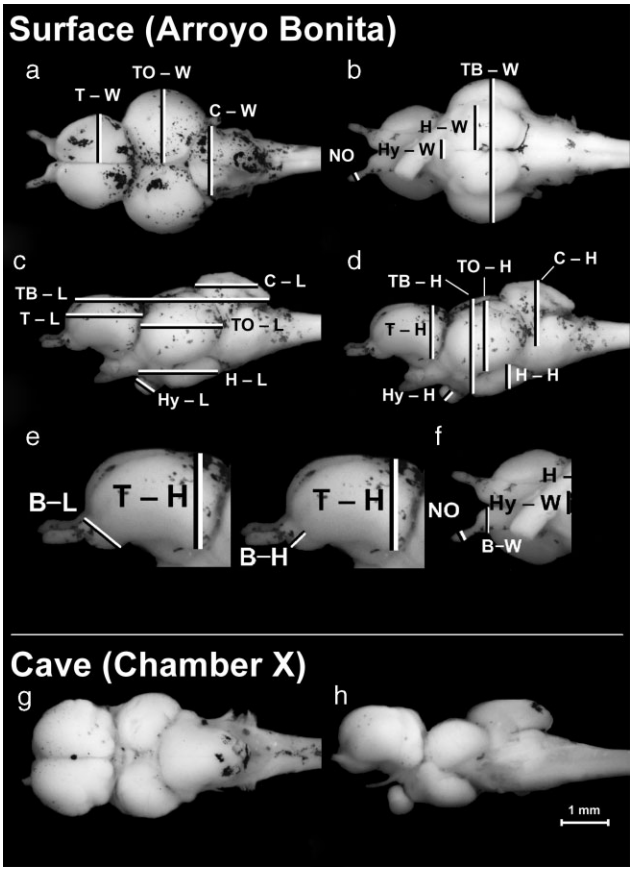

Figure 2 (a–f) Representative brains of fish from a surface habitat (Arroyo Bonita, AB, SL = 35 mm) and (g and h) from the sulphidic cave (Cueva del Azufre, CA-X, SL = 38.4 mm). Linear measurements of brain structures are shown in dorsal (a and g), ventral (b and f) and lateral (c–e, h) views. (e and f) represent enlargements of (b–d) illustrating the olfactory bulb measurements. Note the reduced size of the optic tectum, the enlarged cerebellum and telencephalic lobes in the cave fish compared with the respective brain regions of the surface fish. Rostral is to the left. All structures are shown to the same scale except (e and f) L, length; W, width; H, height; NO, olfactory nerve; TB, total brain; T, telencephalic lobe; TO, optic tectum; C, cerebellum; H, hypothalamus; Hy, hypophysis.

Estimated volumes were corrected for overall size differences with fish standard length and brain mass. First, a size/mass-related variable was derived from a correlation matrix-based principal component analysis on the variables 'standard length' and 'brain mass'. Volumes of different brain regions were then size corrected by a multivariate analysis of covariance (MANCOVA) using the size/mass-related variable as covariate. Likewise, total brain volume and olfactory bulb volume were size corrected by similar ANCOVAs. From the MANCOVA or ANCOVAs, we extracted unstandardized residuals, which were then used as input variables for further analyses.

Total brain and olfactory bulb size were excluded from further multivariate analyses as total brain volume is intercorrelated with volumes of brain regions and as data for olfactory bulbs were not available for all individuals.

We additionally analysed the size of two sensory structures that were assumed to correlate with cave life, that is, the diameter of the olfactory nerve and the eyes. Each of the two variables was size corrected by an ANCOVA using the size/mass-related variable as covariate. Unstandardized residuals from these ANCOVAs were then used as input variables for further analyses.

We tested for differences between sites and sexes for each trait separately. 'Site' and 'sex' were used as fixed factors including the respective interaction term, while size-corrected volumes of the total brain or brain regions, olfactory nerve or eye diameters served as dependent variables. 'Sex' was included because of the well-known sexual dimorphism of the teleost brain (e.g. Bass & Grober, 2001; Kotrschal *et al.*, 2012*a,b*). To illustrate trait differences among sites and between sexes, estimated marginal means (EMMs) were calculated from the respective analytical models (ANOVAs).

In order to compare the six sites (AB, EA, CA-II, CA-V, CA-X, LA) regarding potential effects of light or darkness and presence or absence of H_2S on the size of the respective brain regions, we subjected size-corrected volumes of telencephalic lobes, optic tectum, hypothalamus and cerebellum to an analytical multivariate analysis of variance (MANOVA). Our model included the factors 'light' (present/absent), 'toxicity' (present/absent), 'sex' and all interaction terms.

Comparison of wild-caught and common-garden reared fish

We tested for heritable versus plastic differences in total brain size and size of brain regions between wild-caught and laboratory-reared females from all populations, and thus, calculated an analytical MANOVA (size-corrected brain regions: telencephalic lobes, optic tectum, hypothalamus, cerebellum) or analytical ANOVAs (olfactory nerve, eyes), while 'site' [OX (=AB), EA, CA-II, CA-V, CA-X and LA] and 'rearing condition' (wild-caught vs. lab-reared) were treated as fixed factors including the interaction 'site × rearing condition'.

To obtain a more direct measure of repeatability, that is, broad sense heritability, we conducted intraclass correlation coefficient (ICC) analyses for each dependent variable (size-corrected volumes, olfactory nerve diameter, eye diameter) using the $n = 26$ wild-caught and $n = 26$ lab-reared females (e.g. Bartko, 1966; Riesch *et al.*, 2013).

We additionally tested for broad sense heritability within each site and rearing condition by conducting a discriminant function analysis (DFA). The DFA model was built on the data (size-corrected volumes of brain regions) from wild-caught fish (training dataset; $n = 9$ to 13 females per site, except LA with $n = 4$). We then applied the discriminant functions to the data from the common-garden reared fish (test dataset; $n = 3$ to 5 females per site) and tested for success of correct classification (Hair *et al.*, 1995; Riesch *et al.*, 2011).

All statistical analyses were performed with SPSS 12.0 (SPSS Inc., Chicago, IL, USA).

Table 2 ANOVA results displaying differences between the sites (AB, EA, CA-II, CA-V, CA-X and LA) and/or sex dimorphism of wild-caught fishes

Brain region	Factor	d.f.	Mean square	*F*	*P*	Partial η^2
Olfactory bulbs	Site	5	0.009	2.77	**0.023**	0.13
	Sex	1	0.003	0.91	0.34	0.01
	Site × sex	5	0.003	0.85	0.52	0.04
	Error	89	0.003	–	–	–
Telencephalic lobes	Site	5	1.351	5.42	**<0.001**	0.21
	Sex	1	0.326	1.31	0.25	0.01
	Site × sex	5	0.114	0.46	0.80	0.02
	Error	98	0.249	–	–	–
Optic tectum	Site	5	3.667	5.64	**<0.001**	0.22
	Sex	1	11.174	17.19	**<0.001**	0.14
	Site × sex	5	2.403	3.70	**0.004**	0.15
	Error	98	0.650	–	–	–
Hypothalamus	Site	5	0.304	0.80	0.55	0.04
	Sex	1	0.280	0.74	0.39	0.00
	Site × sex	5	0.105	0.28	0.92	0.01
	Error	97	0.380	–	–	–
Cerebellum	Site	5	15.189	10.97	**<0.001**	0.35
	Sex	1	9.772	7.06	**0.009**	0.06
	Site × sex	5	0.637	0.46	0.80	0.02
	Error	98	1.384	–	–	–
Total brain	Site	5	177.141	3.14	**0.011**	0.13
	Sex	1	725.767	12.87	**0.001**	0.11
	Site × sex	5	112.958	2.00	0.08	0.09
	Error	97	56.395	–	–	–

Dependent variables were size-corrected volumes of olfactory bulbs, telencephalic lobes, optic tectum, hypothalamus, cerebellum and total brain, fixed factors 'site' and 'sex'. *F*-ratios were approximated using Wilks' lambda values. Significant *P*-values are bold.

Results

Brain size variation in wild-caught fish

The volumes of the olfactory bulbs, telencephalic lobes, optic tectum, cerebellum, as well as the total brain differed significantly across sites (Table 2, Supporting Information Table S1). Only presence of light had a significant effect on brain region sizes of the different populations (Table 3A). Size of the optic tectum (Fig. 3b) and the total brain (Fig. 3f) decreased from surface-dwelling populations (AB, EA) to those inhabiting either of the two caves (except CA-V, CA-X). An increase of brain region volumes from surface fish towards cave fish was observed for the olfactory bulbs (Fig. 3e), telencephalic lobes (Fig. 3a) and cerebellum (Fig. 3c; except in fish from LA).

Significant sexual dimorphism was detected for two brain regions and total brain size (Table 2, Fig. 3b, c and f), and males had enlarged optic tecta (especially in CA-V and CA-X; Fig. 3b) and cerebella (Fig. 3c) and larger total brain volumes (Fig. 3f).

The olfactory nerve diameter was similar in all populations ($F_{5,84} = 0.64$, $P = 0.67$; Fig. 4a). In contrast, eye size differed significantly across sites ($F_{5,97} = 3.03$, $P = 0.014$) and exhibited the predicted morphocline with a gradual size decrease from surface habitats to the caves. Specifically, eye size was virtually unchanged in CA-II compared with surface fish from EA, and was then gradually reduced from CA-II towards CA-X (Table S1; Fig. 4b).

Table 3 MANOVA results of size-corrected (residuals) brain region volumes (telencephalic lobes, optic tectum, cerebellum, hypothalamus) used as dependent variables

	Factor	d.f.	*F*	*P*	Partial η^2
A	Light	4	18.81	**<0.001**	0.44
	Toxicity	4	1.95	0.10	0.07
	Sex	4	0.95	0.43	0.03
	Light × toxicity	4	2.19	0.07	0.08
	Light × sex	4	1.33	0.26	0.05
	Toxicity × sex	4	2.28	0.06	0.08
	Light × toxicity × sex	4	1.07	0.37	0.04
B	Site	20	5.59	**<0.001**	0.25
	Rearing condition	4	25.37	**<0.001**	0.56
	Site × rearing condition	20	1.89	**0.013**	0.10

A included wild samples (AB, EA, CA-II, CA-V, CA-X and LA) with 'light', 'toxicity' and 'sex' as fixed factors. B tested lab-reared and wild females with 'site' and 'rearing condition' as fixed factors. *F*-ratios were approximated using Wilks' lambda values. Significant *P*-values are bold.

Heritability versus phenotypic plasticity

Brain region volumes of common-garden reared and wild-caught females showed significant differences depending on 'site' and 'rearing condition' (Table 3B). In contrast to fish from natural populations, lab-reared females displayed similar sizes of both individual brain regions and total brain

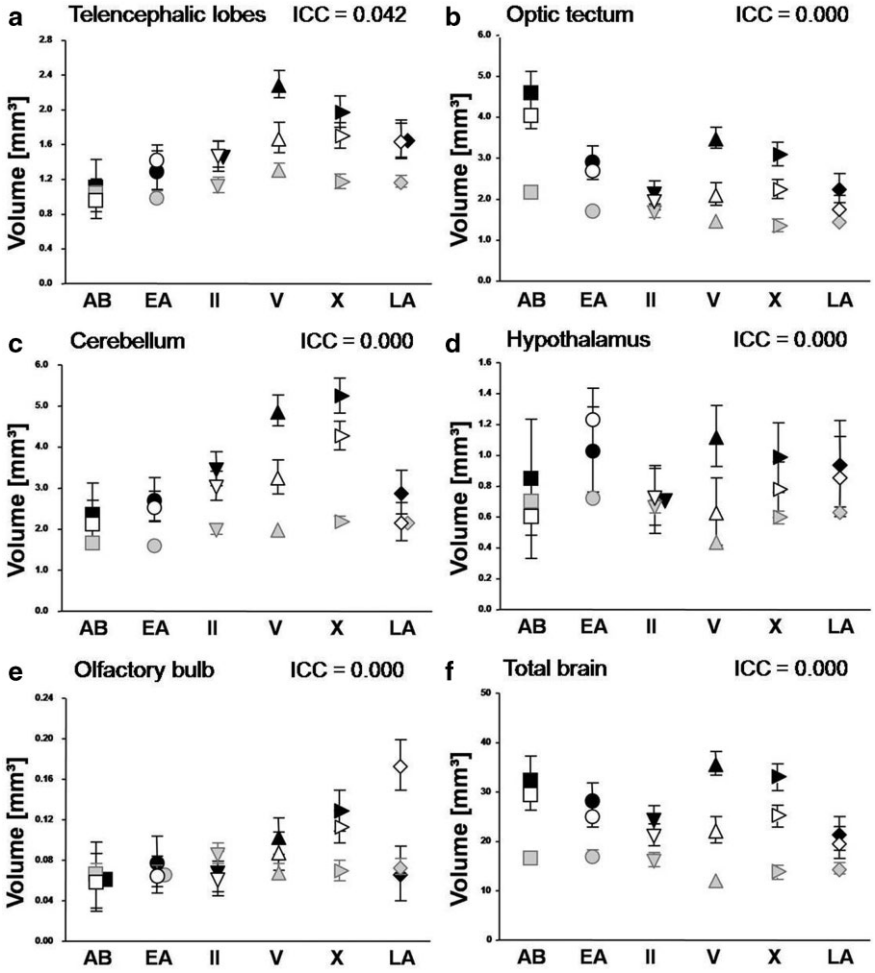

Figure 3 Volumes (EMM ± SEM, size corrected) of (a) telencephalic lobes, (b) optic tectum, (c) cerebellum, (d) hypothalamus, and (e) olfactory bulbs, and (f) total brain of male and female *Poecilia mexicana* from the six sites Arroyo Bonita (AB), El Azufre (EA), cave chamber II (II), chamber V (V), chamber X (X) of the Cueva del Azufre, and Cueva Luna Azufre (LA). Closed black symbols represent males, open symbols represent females; closed grey symbols refer to laboratory-reared females. Intraclass correlation coefficients (ICC) are indicated in the upper right corner of each graph.

regardless of their origin, and sizes were generally smaller than those observed in wild-caught specimens (see interaction effect of 'site × rearing condition' in Table 3B; Fig. 3). Accordingly, ICC analyses revealed that most of the variance was a plastic response to the rearing environment with all ICC values ≤ 0.042 ($P \geq 0.38$ in all cases). The DFA supported these findings as only 19.2% of the lab-reared individuals (test dataset) were correctly assigned to the respective population of origin as quantified by the wild-caught specimens (training dataset).

Eye size differed significantly across sites ($F_{5,81} = 2.40$, $P = 0.044$) independent of rearing conditions ($F_{1,81} = 2.27$, $P = 0.14$). In contrast to phenotypic plasticity of brain regions, the high ICC (ICC = 0.83, $P = 0.017$) suggests trait heritability (Fig. 4b).

Discussion

Heritability versus phenotypic plasticity

We investigated patterns of brain region size variation in a live-bearing fish thriving in habitats with starkly different abiotic conditions to test whether any divergence found in wild fish is mainly caused by evolutionary diversification (i.e. local adaptation) or a result of environmentally induced phenotypic plasticity. Based on previous studies on *P. mexicana* ecotypes, we predicted the strong selective pressures caused by toxic H_2S in EA and CA, food limitation in LA, and darkness in CA and LA, to result in smaller brains. Moreover, the improvement of non-visual senses could be tied to an increase of the respective computational brain regions. Our data from wild-caught fish support both predictions and seemingly provide support for the mosaic evolution hypothesis. However, the results of our common-garden experiment indicate – in accordance with former studies on other fish species (e.g. Kotrschal *et al.*, 1998, 2012*a,b*; Pollen *et al.*, 2007; Gonzalez-Voyer *et al.*, 2009; Gonzalez-Voyer *et al.*, 2010) – that variation in brain region volumes of *P. mexicana* brains is due to phenotypic plasticity and that the above-mentioned ecological factors may thus mainly act on brain (region) development during ontogeny.

Several derived traits in *P. mexicana* from this system have a heritable basis, including enlarged head size, altered body shape and reduced eye size (Tobler *et al.*, 2008*a*), as well as

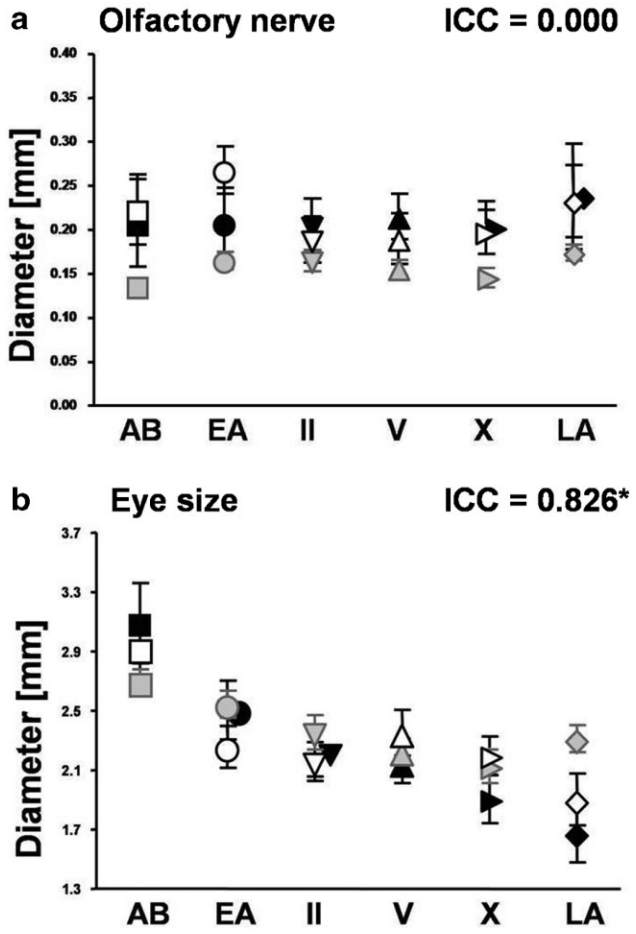

Figure 4 Diameter (EMM ± SEM, size corrected) of (a) the olfactory nerve and (b) the eye of *Poecilia mexicana* specimens from the six sites Arroyo Bonita (AB), El Azufre (EA), cave chamber II (II), chamber V (V), chamber X (X) of the Cueva del Azufre and Cueva Luna Azufre (LA). Closed black symbols represent males, open symbols represent females; grey symbols refer to laboratory-reared females. Intraclass correlation coefficients (ICC) are indicated in the upper right corner of each graph. $^*P = 0.017$.

increased offspring size and the correlated reduced fecundity (Riesch *et al.*, 2009, 2010). So what are the reasons for brain (region) size plasticity? Gonda *et al.* (2013) proposed that brain plasticity itself might be a target of selection. While marine populations of the nine-spined stickleback adapted to high predation risk developed large olfactory bulbs regardless of rearing conditions (no predation vs. predation regime), pond populations showed pronounced phenotypic plasticity by having distinctly larger olfactory bulbs only when experimentally exposed to predation (Gonda *et al.*, 2012). No such habitat-dependent plasticity was found in *P. mexicana*, in which brains and brain regions were similar-sized in all common-garden reared fish. Theoretically, it would be advantageous for *P. mexicana* to retain phenotypic plasticity of brain size if fish still showed considerable migration rates between the different habitats, which would demand for the ability to adjust brain (size) development according to the presence or absence of the stressors darkness and toxic H_2S. Conversely, migration was shown to be very low between habitats differing in presence of at least one abiotic stressor ('light' or 'H_2S'; Plath *et al.*, 2007).

Even though previous studies demonstrated a certain amount of brain size plasticity, it was argued that brain size should be mainly genetically determined because it is of eminent functional importance and as it is an energetically expensive tissue (Gonda *et al.*, 2013). Locally adapted *P. mexicana* in our study system undergo incipient speciation and are in the early stage along the speciation continuum between panmixis and complete reproductive isolation (e.g. Plath *et al.*, 2007, 2013; Tobler *et al.*, 2008*a*, 2009*a*; Plath & Tobler, 2010). Thus, while other traits are already genetically fixed in *P. mexicana*, differences in brain size in wild populations might become genetically fixed only at a later (potentially future) stage of the speciation process.

Overall reduced brain size of laboratory-reared fish was also reported in previous studies on other fish species. First-generation laboratory-reared nine-spined sticklebacks (*Pungitius pungitius*), for example, have between 2% (optic tectum) and 67% (cerebellum) smaller brain regions than wild-caught fish (Gonda *et al.*, 2011) and first-generation laboratory-reared guppies (*P. reticulata*) show smaller telencephala (19%) and optic tecta (17%; Burns *et al.*, 2009). The reasons for overall smaller brains in laboratory-reared fish, as seen in *P. mexicana*, are still unclear. While several authors argued that reduced brain size in laboratory-reared fish might be due to lower structural complexity of the lab environment compared with natural environments (e.g. Kihslinger & Nevitt, 2006*a* for *Oncorhynchus mykiss*; Gonda *et al.*, 2011), studies testing this assumption did not find a tight correlation between the complexity of the lab environment and fish brain size (Kihslinger & Nevitt, 2006*b* for *O. tschawytscha*; Burns *et al.*, 2009 for *P. reticulata*).

Adaptive phenotypic plasticity

Because fish have lifelong growth, it is straightforward to assume that growth of the eyes (i.e. the sensory part) and the optic tectum (i.e. the computational part) should occur in a concerted fashion to ensure proper sensory physiological functions (Zupanc, 2001; Cerveny, Varga & Wilson, 2012). Thus, our finding that eye size differences are heritable when differences in optic tecta are not is puzzling. Studies on cave and surface forms of *Astyanax mexicanus* (e.g. Soares *et al.*, 2004) or Medaka (*Oryzias latipes*) mutants (Ishikawa *et al.*, 1999, 2001) indicate that visual input, that is, the amount of light, positively affects tectum growth and/or maintenance. Although eye size is reduced in cave *P. mexicana*, their eyes are still functional (Körner *et al.*, 2006). It is thus tempting to speculate that laboratory-reared *P. mexicana* developed similar-sized optic tecta because of the presence of functional eyes in surface and cave forms that detected the same amount of visual input in the common-garden set-up. In turn, smaller optic tecta in wild surface fish from sulphidic (murky) waters,

and especially wild cave fish, may be a response to reduced amounts of light or complete darkness. However, visual input is only one factor potentially influencing the size of the optic tectum as studies comparing different social environments (isolated vs. group-reared sticklebacks) also found distinct differences in optic tectum size (Gonda *et al.*, 2009*a*).

Since the brain is a metabolically expensive organ (Isler & van Schaik, 2006; Kotrschal *et al.*, 2013), the reduction of the optic tectum and total brain size in wild cavefish and fish from sulphidic habitats might also be a plastic response to energy-consuming detoxification of H_2S (CA) and low-energy availability (LA; Tobler, 2008). In other words, smaller brains could help reduce energy expenditure in energy-limited sulphidic and cave habitats, where *P. mexicana* are known to also exhibit low body condition (Tobler *et al.*, 2006; Riesch *et al.*, 2010, 2011).

Furthermore, both toxicity and food limitation probably exert a substantial amount of stress on the fish inhabiting CA, EA and LA, which may also affect brain development. Congruently, it was shown that stress due to overcrowding or hypoxia leads to decreased brain growth in *O. tshawytscha* (Kihslinger & Nevitt, 2006*b*), reduced dendritic growth in the optic tectum in *Hemichromis bimaculatus* (Burgess & Coss, 1981) or smaller brains in mormyrids (Chapman & Hulen, 2001). In rats and songbirds, food limitation during ontogenetic development results in a reduction of neural growth and reduced size of brain nuclei (Buchanan *et al.*, 2004; Mirescu, Peters & Gould, 2004). Altogether then, reduced optic tectum size and total brain size in fish from EA, CA and LA may be explained by a combination of less visual input influencing tectum growth and/or maintenance, energetic constraints through H_2S detoxification, and increased stress in the form of toxicity and food limitation.

Sex-specific differences

In our study, *P. mexicana* males exhibited larger optic tecta, cerebella and total brains. Larger brains in males were also shown for nine-spined sticklebacks (Kotrschal *et al.*, 2012*a*) and were explained by greater cognitive demands because of mate attraction and male parental care. In *P. mexicana*, neither males nor females exhibit post-parturiation parental care, but other behavioural differences and different time allocations between *P. mexicana* males and females, as identified for mate acquisition, aggressiveness or foraging behaviour (e.g. Parzefall, 2001; Plath & Tobler, 2010; Köhler *et al.*, 2011; Bierbach *et al.*, 2012) may explain these sex-dependent differences in brain size.

Beside intrinsic sex differences, Kotrschal *et al.* (2012*b*) also demonstrated that brain size variation has a sex-specific plastic component: guppies raised in same-sex groups possessed smaller brains in males and larger optic tecta in females than the respective sex raised in mixed-sex groups. Future studies on *P. mexicana* are warranted to investigate whether a similar sex-specific plasticity of brain size can be identified in the ecotypes of *P. mexicana*.

Conclusions and outlook

Pronounced differences in brain region volumes between *P. mexicana* ecotypes in the wild seemed to fit within the framework of mosaic evolution while our broad sense heritability analysis identified a high amount of phenotypic plasticity. Our study on different ecotypes of *P. mexicana*, therefore, highlights the need for more intraspecific studies on brain evolution investigating brain variation in wild-caught fish and comparing results with those obtained from common-garden reared individuals to provide heritability estimates for any presumed evolutionary changes. Moreover, our results are suggestive of the importance of the factor 'light' on brain development and indicate that brain regions show different ('mosaic'-like) plastic responses, such as decreased optic tectum size and a simultaneously increased cerebellum in CA fish.

We are aware that our study design is only sensitive to differences in outer brain morphology [similar to studies on cichlids (Pollen *et al.*, 2007) and guppies (Kotrschal *et al.*, 2012*a,b*)], so that more fine-scale differences in brain architecture might have gone unnoticed. However, detailed histological studies on brain structure are currently ongoing and will build on the results presented here. Further experimental approaches like long-term translocation experiments – for example, raising wild cave fish (CA or LA) in mesocosms in surface habitats (EA or AB) and vice versa – may provide additional insights into the effects of the factor 'light' and other ecological factors on the ontogenetic development of brain size and architecture in our study species. Finally, future studies will also have to consider potential effects of spatial constraints caused by the neurocranium, which was shown to be another important factor acting upon brain size divergence in fishes (Tsuboi, Gonzalez-Voyer & Kolm, 2014).

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References

Aiello, L.C. & Wheele, P. (1995). The expensive tissue hypothesis – the brain and digestive system in human and primate evolution. *Curr. Anthropol.* **36**, 199–221.

Atwell, D. & Laughlin, S.B. (2001). An energy budget for signalling in the grey matter of the brain. *J. Cereb. Blood Flow Metab.* **21**, 1133–1145.

Bagarinao, T. (1992). Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. *Aquat. Toxicol.* **24**, 1–2. 21–62.

Bartko, J.J. (1966). The intraclass correlation coefficient as a measure of reliability. *Physiol. Rep.* **19**, 3–11.

Barton, R.A. & Harvey, P.H. (2000). Mosaic evolution of brain structure in mammals. *Nature* **405**, 1055–1058.

Bass, A.H. & Grober, M.S. (2001). Social and neural modulation of sexual plasticity in teleost fish. *Brain Behav. Evol.* **57**, 293–300.

Bierbach, D., Klein, M., Sassmannshausen, V., Schlupp, I., Riesch, R., Parzefall, J. & Plath, M. (2012). Divergent evolution of male aggressive behaviour: another reproductive isolation barrier in extremophile poeciliid fishes? *Int. J. Evol. Biol.* **2012**, 1–14.

Buchanan, K.L., Leitner, S., Spencer, K.A., Goldsmith, A.R. & Catchpole, C.K. (2004). Developmental stress selectively affects the song control nucleus HVC in the zebra finch. *Proc. R. Soc. B.* **271**, 2381–2386.

Burgess, J.W. & Coss, R.G. (1981). Short-term juvenile crowding arrests the developmental formation of dendritic spines on tectal interneurons in jewel fish. *Dev. Psychobiol.* **14**, 389–396.

Burns, J.G. & Rodd, F.H. (2008). Hastiness, brain size and predation regime affect the performance of wild guppies in a spatial memory task. *Anim. Behav.* **76**, 911–922.

Burns, J.G., Saravanan, A. & Rodd, F.H. (2009). Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. *Ethology* **115**, 122–133.

Butler, A.B. & Hodos, W. (2005). *Comparative vertebrate neuroanatomy. Evolution and adaptation*. 2nd edn. Hoboken: Wiley-Interscience.

Cerveny, K.L., Varga, M. & Wilson, S.W. (2012). Continued growth and circuit building in the anamniote visual system. *Dev. Neurobiol.* **72**, 328–345.

Chapman, L.J. & Hulen, K.G. (2001). Implications of hypoxia for the brain size and gill morphometry of mormyrid fishes. *J. Zool.(Lond.)* **254**, 461–472.

Charvet, C.J., Striedter, G.F. & Finlay, B.L. (2011). Evodevo and brain scaling: candidate developmental mechanisms for variation and constancy in vertrebrate brain evolution. *Brain Behav. Evol.* **78**, 248–257.

Crispo, E. & Chapman, L.J. (2010). Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. *J. Evol. Biol.* **23**, 2091–2103.

Finlay, B.L. & Darlington, R.B. (1995). Linked regularities in the development and evolution of mammalian brains. *Science* **268**, 1578–1584.

Finlay, B.L., Darlington, R.B. & Nicastro, N. (2001). Developmental structure in brain evolution. *Behav. Brain Sci.* **24**, 298–308.

Fontanier, M.E. & Tobler, M. (2009). A morphological gradient revisited: cave mollies vary not only in eye size. *Environ. Biol. Fish.* **86**, 285–292.

Gonda, A., Herczeg, G. & Merilä, J. (2009*a*). Adaptive brain size divergence in nine-spined sticklebacks (*Pungitius pungitius*)? *J. Evol. Biol.* **22**, 1721–1726.

Gonda, A., Herczeg, G. & Merilä, J. (2009*b*). Habitat-dependent and -independent plastic response to social environment in the nine-spined stickleback (*Pungitius pungitius*) brain. *Proc. R. Soc. B.* **276**, 2085–2092.

Gonda, A., Herczeg, G. & Merilä, J. (2011). Population variation in brain size of nine-spined sticklebacks (*Pungitius pungitius*) – local adaptation or environmentally induced variation? *BMC Evol. Biol.* **11**, 75.

Gonda, A., Välimäki, K., Herczeg, G. & Merilä, J. (2012). Brain development and predation: plastic responses depend on evolutionary history. *Biol. Lett.* **8**, 249–252.

Gonda, A., Herczeg, G. & Merilä, J. (2013). Evolutionary ecology of intraspecific brain size variation: a review. *Ecol. Evol.* **3**, 2751–2764.

Gonzalez-Voyer, A., Winberg, S. & Kolm, N. (2009). Brain structure evolution in a basal vertebrate clade: evidence from phylogenetic comparative analysis of cichlid fishes. *BMC Evol. Biol.* **9**, 238.

Gonzalez-Voyer, A., Kolm, N. & Iwaniuk, A. (2010). Sex, ecology and the brain: evolutionary correlates of brain structure volumes in Tanganyikan cichlids. *PLoS ONE* **5**, e14355.

Hager, R., Lu, L., Rosen, G.D. & Williams, R.W. (2012). Genetic architecture supports mosaic brain evolution and independent brain-body size regulation. *Nat. Commun.* **3**, 1079.

Hair, J.F. Jr., Anderson, R.A., Tatham, R.L. & Black, W.C. (1995). *Multivariate data analysis with readings*. Englewood Cliffs: Prentice Hall.

Hofmann, H.A. (2003). Functional genomics of neural and behavioral plasticity. *J. Neurobiol.* **54**, 272–282.

Hulsey, C.D., Garcia de León, F.J. & Rodiles-Hernández, R. (2006). Micro- and macroevolutionary decoupling of cichlid jaws: a test of Liem's key innovation hypothesis. *Evolution* **60**, 2096–2109.

Ishikawa, Y., Yoshimoto, M., Yamamoto, N. & Ito, H. (1999). Different brain morphologies from different genotypes in a single teleost species, the Medaka (*Oryzias latipes*). *Brain Behav. Evol.* **53**, 2–9.

Ishikawa, Y., Yoshimoto, M., Yamamoto, N., Ito, H., Yasuda, T., Tokunaga, F., Iigo, M., Wakamatsu, Y. & Ozato, K. (2001). Brain structures of a Medaka mutant, *el* (eyeless), in which eye vesicles do not evaginate. *Brain Behav. Evol.* **58**, 173–184.

Isler, K. & van Schaik, C.P. (2006). Metabolic costs of brain size evolution. *Biol. Lett.* **2**, 57–560.

Jacyna, S. (2009). The most important of all the organs: Darwin on the brain. *Brain* **132**, 3281–3487.

Kihslinger, R.L. & Nevitt, G.A. (2006*a*). Early rearing environment impacts cerebellar growth in juvenile salmon. *J. Exp. Biol.* **209**, 504–509.

Kihslinger, R.L. & Nevitt, G.A. (2006*b*). Environmental rearing conditions produce forebrain differences in wild Chinook salmon *Oncorhynchus tshawytscha*. *Comp. Biochem. Physiol. A* **145**, 145–151.

Körner, K.E., Schlupp, I., Plath, M. & Loew, R. (2006). Spectral sensitivity of mollies: comparing surface- and cave-dwelling Atlantic mollies, *Poeciila mexicana*. *J. Fish Biol.* **69**, 54–65.

Kotrschal, A., Räsänen, K., Kristjánsson, B.K., Senn, M., Kolm, N. & Iwaniuk, A. (2012*a*). Extreme sexual brain size dimorphism in sticklebacks: a consequence of the cognitive challenges of sex and parenting? *PLoS ONE* **7**, e30055.

Kotrschal, A., Rogell, B., Maklakov, A.A. & Kolm, N. (2012*b*). Sex-specific plasticity in brain morphology depends on social environment of the guppy, *Poecilia reticulata*. *Behav. Ecol. Sociobiol.* **66**, 1485–1492.

Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brännström, I., Immler, S., Maklakov, A.A. & Kolm, N. (2013). Artificial selection on relative brain size in the Guppy reveals costs and benefits of evolving a larger brain. *Curr. Biol.* **23**, 168–171.

Kotrschal, K., van Staaden, M. & Huber, R. (1998). Fish brains: evolution and environmental relationships. *Rev. Fish Biol. Fish.* **8**, 373–408.

Köhler, A., Hildenbrand, P., Schleucher, E., Riesch, R., Arias-Rodriguez, L., Streit, B. & Plath, M. (2011). Effects of male sexual harassment on female time budgets, feeding behavior, and metabolic rates in a tropical livebearing fish (*Poecilia mexicana*). *Behav. Ecol. Sociobiol.* **65**, 1513–1523.

Liem, K.F. (1978). Modularity multiplicity in the functional repertoire of the feeding mechanism in Cichlid fishes. *J. Morphol.* **158**, 323–360.

Mirescu, C., Peters, J.D. & Gould, E. (2004). Early life experience alters response of adult neurogenesis to stress. *Nat. Neurosci.* **7**, 841–846.

Nottebohm, F. (1981). A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. *Science* **214**, 1368–1370.

Parzefall, J. (1970). Morphologische Untersuchungen an einer Höhlenform von *Mollienesia sphenops* (Pisces, Poeciliidae). *Z. Morphol. Tiere* **68**, 323–342.

Parzefall, J. (2001). A review of morphological and behavioural changes in the cave molly, *Poecilia mexicana*, from Tabasco, Mexico. *Environ. Biol. Fish.* **62**, 263–275.

Plath, M. & Tobler, M. (2010). Subterranean fishes of Mexico. In *Biology of subterranean fishes*: 281–330. Trajano, E., Bichuette, M.E. & Kapoor, B.G. (Eds). Enfield: Science Publishers.

Plath, M., Hauswaldt, J.S., Moll, K., Tobler, M., García de León, F.J., Schlupp, I. & Tiedemann, R. (2007). Local adaptation and pronounced genetic differentiation in an extremophile fish, *Poecilia mexicana*, inhabiting a Mexican cave with toxic hydrogen sulphide. *Mol. Ecol.* **16**, 967–976.

Plath, M., Riesch, R., Oranth, A., Dzienko, J., Karau, N., Schießl, A., Stadler, S., Wigh, A., Zimmer, C., Arias-Rodriguez, L., Schlupp, I. & Tobler, M. (2010). Complementary effect of natural and sexual selection against immigrants maintains differentiation between locally adapted fish. *Naturwissenschaften* **97**, 769–774.

Plath, M., Pfenninger, M., Lerp, H., Riesch, R., Eschenbrenner, C., Slattery, P.A., Bierbach, D., Herrmann, N., Schulte, M., Arias-Rodriguez, L., Indy, J.R., Passow, C. & Tobler, M. (2013). Genetic differentiation and selection against migrants in evolutionarily replicated extreme environments. *Evolution* **67**, 2647–2661.

Pollen, A.A., Dobberfuhl, A.P., Scace, J., Igulu, M.M., Renn, S.C., Shumway, C.A. & Hofmann, H.A. (2007). Environmental complexity and social organization sculpt the brain in lake Tanganyikan cichlid fish. *Brain Behav. Evol.* **70**, 21–39.

Riesch, R., Tobler, M., Schlupp, I. & Plath, M. (2009). Offspring number in a livebearing fish (*Poecilia mexicana*, Poeciliidae): reduced fecundity and reduced plasticity in a population of cave mollies. *Environ. Biol. Fishes* **84**, 89–94.

Riesch, R., Plath, M. & Schlupp, I. (2010). Toxic hydrogen sulfide and dark caves: life-history adaptations in a livebearing fish (*Poecilia mexicana*, Poeciliidae). *Ecology* **91**, 1494–1505.

Riesch, R., Plath, M. & Schlupp, L. (2011). Toxic hydrogen sulphide and dark caves: pronounced male life-history divergence among locally adapted *Poecilia mexicana* (Poeciliidae). *J. Evol. Biol.* **24**, 596–606.

Riesch, R., Martin, R.A., Langerhans, R.B. (2013). Predation´s role in life-history evolution of a livebearing fish and a test of the Trexler-DeAngelis model of maternal provisioning. *Amer. Nat.* **181**, 78–93.

Soares, D., Yamamoto, Y., Strickler, A.G. & Jeffery, W.R. (2004). The lens has a specific influence on optic nerve and tectum development in the blind cavefish *Astyanax*. *Dev. Neurosci.* **26**, 308–317.

Striedter, G.F. (2005). *Principles of brain evolution*. Sunderland: Sinauer Associates.

Tobler, M. (2008). Divergence in trophic ecology characterizes colonization of extreme habitats. *Biol. J. Linn. Soc.* **95**, 517–528.

Tobler, M. (2009). Does a predatory insect contribute to the divergence between cave- and surface-adapted fish populations? *Biol. Lett.* **5**, 506–509.

Tobler, M. & Plath, M. (2011). Living in extreme environments. In *Ecology and evolution of poeciliid fishes*: 120–127. Evans, P., Pilastro, A. & Schlupp, I. (Eds). Chicago and London: The University of Chicago Press.

Tobler, M., Schlupp, I., Heubel, K.U., Riesch, R., Garcia de León, F.J., Giere, O. & Plath, M. (2006). Life on the edge: hydrogen sulphide and the fish communities of a Mexican cave and surrounding waters. *Extremophiles* **10**, 577–585.

Tobler, M., DeWitt, T.J., Schlupp, I., García de León, F.J., Herrmann, R., Feulner, P.G.D., Tiedemann, R. & Plath, M. (2008*a*). Toxic hydrogen sulfide and dark caves: phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. *Evolution* **62**, 2643–2659.

Tobler, M., Riesch, R., García de León, F.J., Schlupp, I. & Plath, M. (2008*b*). A new and morphologically distinct population of cavernicolous *Poecilia mexicana* (Poeciliidae: Teleostei). *Environ. Biol. Fish.* **82**, 101–108.

Tobler, M., Riesch, R. & Tobler, C.M. & Plath, M. (2009*a*). Compensatory behavior in response to sulfide-induced hypoxia affects time budgets, feeding efficiency, and predation risk. *Evol. Ecol. Res.* **11**, 935–948.

Tobler, M., Riesch, R., Tobler, C.M., Schulz-Mirbach, T. & Plath, M. (2009*b*). Natural and sexual selection against immigrants maintains differentiation among micro-allopatric populations. *J. Evol. Biol.* **22**, 2298–2304.

Tobler, M., Coleman, S.W., Perkins, B.D. & Rosenthal, G.G. (2010). Reduced opsin gene expression in a cave-dwelling fish. *Biol. Lett.* **6**, 98–101.

Tobler, M., Palacios, M., Chapman, L.J., Mitrofanov, I., Bierbach, D., Plath, M., Arias-Rodriguez, L., García de León, F.J. & Mateos, M. (2011). Evolution in extreme environments: replicated phenotypic differentiation in livebearing fish inhabiting sulfidic springs. *Evolution* **65**, 2213–2228.

Trajano, E. (1994). Comparative study of the brain and olfactory organ of the troglobitic catfish *Pimelodella kronei* (Ribeiro 1907), and its putative ancestor *P. transitoria* (Ribeiro 1912) (Siluriformes, Pimelodidae). *Trop. Zool.* **7**, 145–160.

Tsuboi, M., Gonzalez-Voyer, A. & Kolm, N. (2014). Phenotypic integration of brain size and head morphology in Lake Tanganyika Cichlids. *BMC Evol. Biol.* **14**, 39.

de Winter, W. & Oxnard, C.E. (2001). Evolutionary radiations and convergences in the structural organization of mammalian brains. *Nature* **409**, 710–714.

Yopak, K.E., Lisney, T.J., Darlington, R.B., Collin, S.P., Montgomery, J.C. & Finlay, B.L. (2010). A conserved pattern of brain scaling from sharks to primates. *Proc. Natl. Acad. Sci. USA* **107**, 12946–12951.

Zupanc, G.K. (2001). Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish. *Brain Behav. Evol.* **58**, 250–275.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Descriptive statistics of raw measurements of eye size, standard length, brain mass, olfactory nerve diameter, olfactory bulb distances, brain area distances and not size-corrected volumina of male and female *Poecilia mexicana* from (A) surface and (B) cave habitats. *N* and SL for the determination of the mean values for the eye diameter are included. SL, standard length; AB, Arroyo Bonita; EA, El Azufre I; CA-II, CA-V, CA-X, chambers of the Cueva del Azufre; LA, Cueva Luna Azufre. Units of all diameter, length (L), width (W) and height (H) measurements are in mm, those of brain mass in mg, and those of all volume (V) measurements in mm^3.