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Environmental Complexity and Social Organization Sculpt the Brain in Lake Tanganyikan Cichlid Fish

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Environmental complexity · Habitat complexity · Social organization · Fish · Cichlid · Evolution · Brain · Ecology

Abstract
Complex brains and behaviors have occurred repeatedly within vertebrate classes throughout evolution. What adaptive pressures drive such changes? Both environmental and social features have been implicated in the expansion of select brain structures, particularly the telencephalon. East African cichlid fishes provide a superb opportunity to analyze the social and ecological correlates of neural phenotypes and their evolution. As a result of rapid, recent, and repeated radiations, there are hundreds of closely-related species available for study, with an astonishing diversity in habitat preferences and social behaviors. In this study, we present quantitative ecological, social, and neuroanatomical data for closely-related species from the (monophyletic) Ectodini clade of Lake Tanganyikan cichlid fish. The species differed either in habitat preference or social organization. After accounting for phylogeny with independent contrasts, we find that environmental and social factors differentially affect the brain, with environmental factors showing a broader effect on a range of brain structures compared to social factors. Five out of seven of the brain measures show a relationship with habitat measures. Brain size and cerebellar size are positively correlated with species number (which is correlated with habitat complexity); the medulla and olfactory bulb are negatively correlated with habitat measures. The telencephalon shows a trend toward a positive correlation with rock size. In contrast, only two brain structures, the telencephalon and hypothalamus, are correlated with social factors. Telencephalic size is larger in monogamous species compared to polygamous species, as well as with increased numbers of individuals; monogamy is also associated with smaller hypothalamic size. Our results suggest that selection or drift can act independently on different brain regions as the species diverge into different habitats and social systems.

Introduction

Comparative studies can help reveal which neural phenotypes, such as relative forebrain size, have been selected as functional units during brain evolution by identifying those variables that best explain changes in phenotype across species [Harvey, 1991]. Two models have been proposed to explain how brains evolve. The adaptationist model, known as mosaic evolution, suggests that

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the brain contains functionally distinct regions mediating particular sets of behaviors [Barton and Harvey, 2000]. Selection on a specific set of behaviors should favor a change localized to the brain region mediating that behavior. The developmental constraints model, on the other hand, recognizes that a common set of genes and developmental processes may regulate the development of a range of functional regions. In this model, selection for a change in any single brain structure would cause the brain to change as a whole unit [Jerison, 1991; Finlay and Darlington, 1995; Finlay et al., 2001]. In addition to these two models, random drift can also underlie differences in brain structure. A recent synthesis provides support for these different processes, suggesting that both mosaic evolution and developmental constraints play fundamental roles in driving brain/behavior changes [Striedter, 2005].

Perhaps because it is more consistent with the adaptationist world view, the mosaic model has often been applied to explain correlations between brain structure size and ecological properties. As a result, the role of developmental constraints has been neglected. For example, the large primate neocortex has been linked to increased visual demands related to diet and (diurnal) foraging behavior [Milton, 1988; Harvey and Krebs, 1990]. Barton [1996] showed that the degree of frugivory correlates with an increase in neocortex size, independently of social group size, and that diurnal primates have larger neocortices than nocturnal primates. In birds, the frequency of feeding innovations described in bird species correlates with the size of the forebrain [Lefebvre et al., 1997; Timmermans et al., 2000]. Changes in hippocampal size relative to spatial memory specializations in food-storing birds, kangaroo rats, and other species also strongly imply mosaic evolution [Sherry et al., 1992; Jacobs and Spencer, 1994; Healy and Krebs, 1996; Reboreda et al., 1996; Lucas et al., 2004]. [However, this same pattern is not observed among food-storing and non-food-storing woodpeckers; Volman et al., 1997.] Recently, Safi and Dechmann [2005] used wing area as a qualitative proxy for habitat complexity in bats, and found positive correlations with hippocampal size and the size of the midbrain auditory region: the inferior colliculus.

The mosaic evolution model has also been applied to social variables, although quantifying social differences has proved controversial. As with environmental factors, social factors also correlate with changes in the size of brain structures. In primates, neocortex size correlates with social group size, a proxy for social complexity, along with group range [Sawaguchi, 1988; Barton, 1996; Reader and Laland, 2002]. The size of song repertoires in oscine birds correlates positively with the volume of the forebrain vocal area HVC [Devoogd et al., 1993]. Burish et al. [2004] suggested that the fraction of the avian brain devoted to the telencephalon increases with group size, leading to the speculation that the telencephalon 'may be an anatomical substrate for social complexity.' [See Beauchamp and Fernandez-Juricic, 2004, however, for a different conclusion, using a different dataset.] Although the Burish et al. data (not corrected for phylogenetic confounds) shows a significant increase in telencephalic expansion for the most complex, but qualitatively defined 'transactional' category compared to other categories, no significant difference was found between solitary (territorial) birds and covey birds, or between solitary and colonial birds. In a functional study of four bird species (also not corrected for phylogenetic confounds), Goodson et al. [2005] found that immediate early gene responses in brain areas implicated in social arousal and dominance-related behaviors inversely correlated with the degree of sociality. These observations are consistent with the 'social brain hypothesis' that telencephalon expansion in the brain is largely an adaptation to the pressures of increasingly complex social cues [Byrne and Whiten, 1988; Dunbar, 1992; Barton and Dunbar, 1997]. As group size increases, the number of possible social cues and responses increases as well.

As described above, primate complex behaviors ranging from foraging for high-quality foods to social intelligence have been linked to the disproportionate enlargement of the neocortex. Assuming adaptive evolution has been important in these processes, what forces play a greater role in driving brain evolution: ecological or social factors or both? Because of phylogenetic confounds and a limited number of primate species available for study, the question is difficult to resolve in this system.

To better understand the relative contributions of ecological and social forces as well as developmental constraints to vertebrate brain evolution it would be best to conduct the analysis on closely-related species that live in diverse habitats and exhibit diverse social systems. Fishes are a perfect group for such studies. Many of the same social and physical gradients thought to have influenced primate brain evolution are present in fish [Bshary et al., 2002]. Ecological constraints in fish might similarly require special foraging skills and sophisticated spatial maps to navigate complex reef or rocky habitats. Additionally, many species of fish live in social groups of varying size and exhibit an astonishing range of parental care ranging from simple (no care) to complex (e.g., coopera-

tive breeding, the highest level of parental care known in fish, where several generations live together and older siblings help raise the young) [Taborsky, 2001; Bshary et al., 2002]. Individual recognition, dominance hierarchies, cooperation, and triadic interactions have been documented [Balshine and Lotem, 1998; Barlow, 2000; Taborsky, 2001].

Cichlid fishes from east Africa's Great Lakes region are an ideal system in which to study ecological and social correlates of neural phenotypes. First, cichlids have experienced the most rapid and extensive adaptive radiations known for vertebrates, resulting in hundreds of species within tens of thousands to a few million years [Salzburger et al., 2002; Verheyen et al., 2003; Kocher, 2004]. In contrast, the long evolutionary time courses of other vertebrate groups make isolating salient selection pressures more difficult [Harvey, 1991]. Second, despite their phenotypic diversity, east African cichlid species are remarkably similar genetically [Salzburger et al., 2002; Renn et al., 2004]. Third, the extraordinary behavioral diversity exhibited by related species allows for comparison across large social and physical gradients [Goodwin et al., 1998; Balshine-Earn and Earn, 1998; Kocher, 2004]. Lastly, because variability of traits across closely-related species is expected to be limited by similar developmental constraints [Harvey, 1991; Clark et al., 2001], discovering such variability for a trait across closely related species might indicate that selection or drift could have operated on that trait.

Previous comparative studies of cichlids from all three African lakes (Victoria, Tanganyika, Malawi) showed that the sizes of brain structures can be related to qualitative categories of physical environments [van Staaden et al., 1995; Huber et al., 1997]. These authors linked telencephalic size to habitat, optic tectum/midbrain size to feeding habits, and dorsal medulla size to trophic tactic. The strength of these studies was the large number of species used for comparison and the multivariate analyses of the brain variations found relative to the ecological variables. However, as these studies relied almost entirely on one (or very few) brains per species obtained from museum specimens, some of the measured variability might be the result of small sample numbers and artifacts due to inconsistent tissue fixation of the museum specimens.

In the present study, we measured brain size and the size of major brain structures of seven closely-related Tanganyikan cichlid species within the monophyletic Ectodini clade differing either in habitat preference or social organization. We also quantitatively measured select properties of the physical and social environment that might be expected to have influenced brain evolution. The monophyly of the Ectodini clade has been demonstrated repeatedly [Nishida, 1991; Sturmbauer and Meyer, 1993; Sturmbauer et al., 1994; Kocher et al., 1995; Sültmann et al., 1995; Takahashi et al., 1998] and phylogenetic relationships within the clade have been largely resolved [Koblmüller et al., 2004].

The thirty-five species in the Ectodini clade are renowned for their behavioral diversity of habitat preference and social behaviors [Barlow, 2000], and are uniquely suited to extend our understanding of the evolution of vertebrate social organization. According to a consensus phylogeny, the clade contains four pair-wise replicates, or contrasts, of monogamous and polygamous species [Koblmüller et al., 2004]. In this study we examine three polygamous and four monogamous species representing two transitions between social systems. Although monogamy is thought to be the ancestral state in cichlids [Goodwin et al., 1998], the multiple transitions between monogamy (with biparental care) and polygamy (with maternal care) suggest that such traits may have evolved independently multiple times in the Ectodini clade.

Materials and Methods

Choice of Species

We chose Lake Tanganyika because of the wide range of social systems present and excellent water clarity. We examined seven Ectodine species differing among habitat preference and social organization: *Xenotilapia ochrogenys, Enantiopus melanogenys, Xenotilapia bathyphila, Xenotilapia boulengeri, Xenotilapia flavipinnis, Xenotilapia spiloptera,* and *Asprotilapia leptura* (fig. 1). The first five species are sand-dwellers; *X. spiloptera* lives in intermediate habitats, and *A. leptura* is a rock-dweller. The first three species are polygamous; the remaining four are monogamous [Konings and Dieckhoff, 1992; Konings, 1996; Hermann, 1996].

We quantified habitat properties and collected specimens during three field seasons (1998, 2003 and 2004) at Kigoma Bay, Tanzania (from 4°52′ S, 29°37′ E to 4°56′ S, 29°35′ E). Using SCUBA, we delineated quadrat borders around focal species using a 20 meter weighted rope shaped into a 5m x 5m square. Typically, 3–4 divers examined 1–2 quadrats per day in calm water (between 9:00 12:00 h; ca. 30–60 min per quadrat). We measured both social properties (number of species and number of individual fish per quadrat, and number of adult conspecifics for each focal species) and physical properties of the environment (depth, slope, surface roughness, and rock size) in 38 quadrats. To obtain representative measures of environmental characteristics for each focal species, we averaged across all quadrats in which a given species was found. Surface roughness (rugosity) was measured three times per quadrat in random locations. Rugosity, a standard measure used to assess coral reef topography, is defined as the ratio of

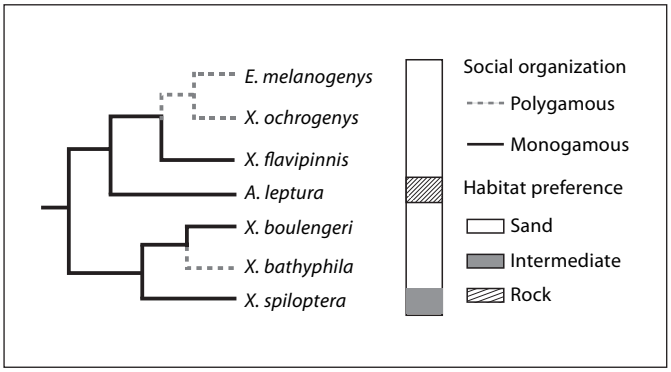

Fig. 1. Evolutionary relationships and associated character traits of the seven cichlid species used in this study. All species belong to the Ectodini clade, which is endemic to Lake Tanganyika. Social organization – polygamous: dashed gray lines; monogamous: black lines; habitat preference – sand-dwellers: white box; intermediate: gray box; rock-dwellers: hatched box [Brichard, 1989; Konings and Dieckhoff, 1992; Hermann, 1996; Konings, 1996]. Phylogenetic tree is adapted from the consensus tree provided in Koblmüller et al. [2004].

actual surface distance to linear distance [Luckhurst and Luckhurst, 1978; García-Charton and Pérez-Ruzafa, 2001]. When rocks were present, we determined rock volume by calculating maximum length, width, and height of five randomly selected rocks in each quadrat. The cube root of the resulting volume estimate was used to compute average rock size. In areas of large fused rocks, the rock boundary was defined by the fissures. When fused rocks extended beyond the quadrat bounds, length and width measures were continued to the rock edge.

Neuroanatomy

Comparing the size of brain structures across species varying in size requires choosing a reference variable [Harvey and Krebs, 1990]. In the past, brain size and brain structure sizes were normalized to body size according to allometric relationships [Jerison, 1991]. However, because body size can vary within a species with respect to sex, season and male reproductive phenotypes [Harvey and Krebs, 1990; Bass and McKibben, 2003], most recent work has used brain size to control for allometric relationships and individual variation [Burish et al., 2004]. This approach can also help evaluate the role of developmental constraints in brain evolution by determining how much of the variation in the size of a particular brain structure is explained by overall brain size alone [Finlay and Darlington, 1995; Finlay et al., 2001]. A second method calculates residuals from such absolute size comparisons, enabling one to explore relationships between brain structure size and ecology [Barton, 1996]. A third method compares the fractions of the whole brain devoted to a particular region [Clark et al., 2001]. Each method has its limitations. Absolute size comparisons tend to highlight covariances [Burish et al., 2004]; residuals depend on cross-species fits [Clark et al., 2001]; and fractions impose an isometric scaling expectation on brain parts [Barton and Dunbar, 1997]. We therefore decided to use all three measures in this study.

To control for sex and reproductive status, only mature males were used. Most specimens were wild-caught (caught in Kigoma Bay: n = 56; purchased from fish dealers: n = 6). Additional animals were obtained from laboratory-reared stocks (n = 6). Fish were anesthetized in MS-222 (200 μg/l), and standard length, width, height and mass were measured. The fish were perfused with a 4% paraformaldehyde, 1% glutaraldehyde solution in phosphate buffer. Brains and gonads were stored in fixative at 4°C. Brains from wild-caught samples were measured within two months after dissection (due to an extended period of fieldwork); all other specimens were measured within a week of dissection. There was no difference between wild-caught and lab-reared fish of the same species with respect to brain regions (e.g., relative telencephalic size in *E. melanogenys*, t test: $t = -0.541$, $p = 0.597$). Although all brain measures reported here were obtained by the same observer with very little variability, it was not always possible to do so blindly. To assess a potential observer bias, 24 brains (from five species) were coded and measurements were conducted blindly by two individuals. Variability was small (coefficient of variation, CV <5% for whole brain, cerebellum, and midbrain; CV <10% for olfactory bulb, hypothalamus, and telencephalon; CV = 16% for dorsal medulla) and random, i.e., none of the observers systematically over- or underestimated any of the brain areas.

Brains were photographed through a dissecting microscope (Zeiss) using a digital camera and accompanying software (Zeiss Axiovision mrc) (fig. 2). For dorsal and ventral views, we ensured that the brain was symmetrically positioned such that one hemisphere did not appear larger than the other based on perspective. All lateral images were taken of the right hemisphere. For paired structures, only the width of the right hemisphere was measured. The volume V of brain structures was determined according to an ellipsoid model [van Staaden et al., 1995; Huber et al., 1997; Wagner, 2001a, b; Lisney and Collin, 2006; Lisney et al., 2007]:

$$V = (L \times W \times H)\ \pi/6$$

For paired structures such as the telencephalon, the estimated volume of the structure was doubled. The width W was defined as the greatest distance enclosed by a given structure and perpendicular to the anatomical midline. The widths of the telencephalon, midbrain, cerebellum, dorsal medulla and whole brain were measured from a dorsal image of the brain (fig. 2A). The widths of the olfactory bulb, hypothalamus and hypophysis were measured from a ventral image of the brain (fig. 2B). The length L of a structure was defined as the greatest distance enclosed by a structure and parallel to the estimated projection of the brain; the height H as the greatest distance enclosed by the structure and perpendicular to the estimated projection of the brain. To determine the height of the hypothalamus, we removed nerves caudal to the midbrain/tectum to find the most dorsal part of the hypothalamus. The lengths and heights of all structures were measured from a lateral image of the brain (fig. 2C, D). Because the ventral boundary of the cerebellum is not always visible from a lateral view, we used the midpoint of the midbrain height as a consistent approximation. Additionally, length (from the caudal edge of the medulla to the rostral edge of the telencephalon) and height of the whole brain (from the ventral edge of the hypothalamus to the dorsal edge of the midbrain) were measured from a lateral image and its width (measured across the two optic tecta) determined from a dorsal image. The volume estimates for whole brain and six brain regions, averaged for each species, are shown in table 1.

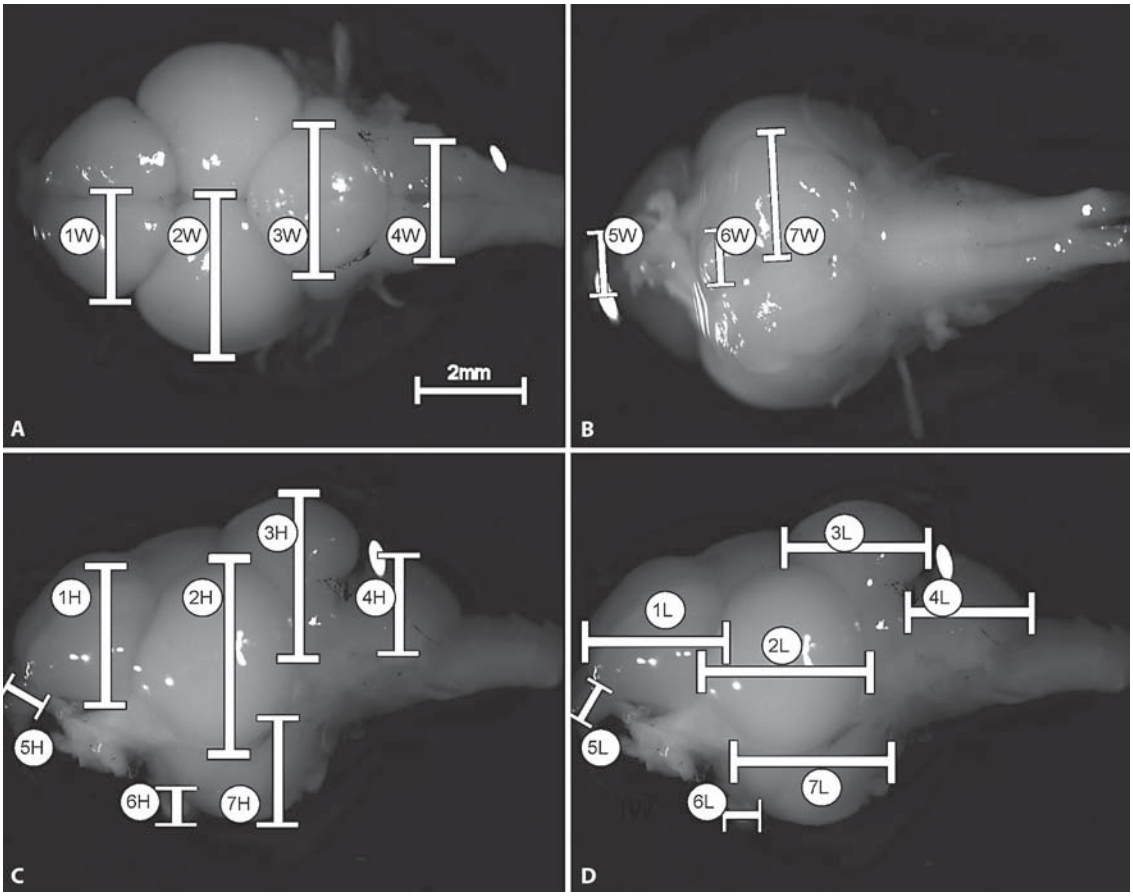

Fig. 2. Illustration of the measurements taken from **A** dorsal, **B** ventral, and **C**, **D** lateral images to determine the size of various brain structures. W, H and L refer to width, height and length, respectively. 1: telencephalon; 2: optic tectum/midbrain; 3: cerebellum; 4: dorsal medulla; 5: olfactory bulb; 6: pituitary; 7: hypothalamus. The brain shown is from *E. melanogenys*.

Estimating Body and Brain Mass

Because not all fish could be weighed reliably in the field, we developed an approximation for body mass and applied it to all samples. We used an ellipsoid model, as described above, to approximate fish volume V. Fish density was assumed to be 1 g/ml. Standard length was defined as the distance from start of the caudal fin to the rostral tip of the mouth; width and height as the maximum horizontal and vertical distances enclosing the fish. We validated this approach by calculating the linear regression of the ellipsoid model against the actual body mass of 55 fish weighed in the lab (slope = 1.05, $R^2 = 0.978$; $p < 0.0001$; n = 55).

Brains were collected from 68 individuals representing the 7 focal species. The nerves were trimmed close to the brain stem and the mass of 62 of the brains was recorded. The first six brains were sectioned before recording the mass. In order to include the first six samples in the data set, we used an ellipsoid model, with the length, width and height as inputs to estimate brain mass. Note that the dorsal edge of the optic tectum was used as the upper bound of height, because the placement of the cerebellum varied across species. Linear regression analysis showed that brain mass can be remarkably well approximated by an ellipsoid across the seven species (slope = 1.23, $R^2 = 0.946$; $p < 0.0001$; n = 62), which is consistent with previous work [van Staaden et al., 1995; Huber et al., 1997; Wagner, 2001a, b; Lisney and Collin, 2006; Lisney et al., 2007]. Because the surface of the brain is not a smooth convex ellipsoid, but also includes concave portions and rapid changes in curvature in between structures, the ellipsoid model systematically overestimated the volume of the brain. We therefore used the slope of the regression as a correction factor for total brain volume only:

$$\text{Brain volume} = (L \times W \times H)\,\pi/(6 \times 1.23)$$

Similarly, we examined how well the ellipsoid model that was applied to gross measures approximated the size of certain brain structures, such as the telencephalon and mid-brain across species. We measured the volumes of three brain areas and the entire brain from histological sections from males of three species: *X. flavipinnis* (n = 2), *X. ochrogenys* (n = 2) and *A. leptura* (n = 2 for optic tectum, cerebellum; n = 3 for telencephalon). We found significant linear relationships between the gross brain mea-

Table 1. Average volume estimates ± SE (in mm^3) for whole brain and six brain regions of seven Ectodini species

Brain area	Species						
	X. ochrogenys	*E. melanogenys*	*X. bathyphila*	*X. flavipinnis*	*X. boulengeri*	*X. spiloptera*	*A. leptura*
Whole brain							
Mass	86.77 ± 2.64	116.06 ± 5.63	82.6 ± 12.16	58.0 ± 2.21	157.3 ± 9.95	73.4 ± 5.33	83.21 ± 2.461
N	14	14	3	9	8	4	10
Vol.[a]	134.72 ± 3.99	199.47 ± 9.26	130.3 ± 16.57	93.76 ± 3.83	229.83 ± 11.99	125.79 ± 8.45	133.87 ± 5.02
N	14	17	3	9	8	6	11
Sect.[b]	48.3 ± 4.60			32.75 ± 2.65			49.27 ± 5.17
Vol.	2			2			2
Ell. Mod.[c]	91.87 ± 2.76	116.21 ± 5.26	89.21 ± 15.70	59.39 ± 2.44	154.81 ± 9.02	76.07 ± 4.27	78.40 ± 2.77
Adj.[d]	55.89 ± 2.76	75.55 ± 5.26	53.74 ± 15.70	29.66 ± 2.44	106.73 ± 9.02	43.13 ± 4.27	45.01 ± 2.77
Telencephalon							
Vol.	24.24 ± 0.73	27.45 ± 1.57	22.18 ± 5.53	17.96 ± 0.92	45.05 ± 3.52	20.87 ± 0.52	28.18 ± 1.19
N	14	16	3	9	8	6	11
Sect.	7.25 ± 0.35			5.3 ± 0.56			8.43 ± .90
Vol.	2			2			3
Ell. Mod.	12.69 ± 0.39	14.39 ± 0.82	11.61 ± 2.90	9.40 ± 0.48	23.59 ± 1.84	10.93 ± 0.27	14.76 ± 0.62
Adj.	7.69 ± 0.39	8.97 ± 0.82	6.88 ± 2.90	5.21 ± 0.48	15.92 ± 1.84	6.36 ± 0.27	9.25 ± 0.62
Cerebellum							
Vol.	13.22 ± 0.60	24.48 ± 1.05	13.68 ± 1.22	8.58 ± 0.64	25.98 ± 1.48	13.36 ± 0.71	17.73 ± 0.45
N	14	16	3	8	8	6	11
Sect.	5.85 ± 0.95			4.6 ± 0.06			8.93 ± 0.27
Vol.	2			2			2
Ell. Mod.	6.92 ± 0.32	12.82 ± 0.55	7.16 ± 0.64	4.49 ± 0.33	13.61 ± 0.78	7.00 ± 0.37	9.28 ± 0.24
Adj.	6.35 ± 0.32	10.80 ± 0.55	6.53 ± 0.64	4.52 ± 0.33	11.39 ± 0.78	6.41 ± 0.37	8.13 ± 0.24
Midbrain							
Vol.	45.40 ± 1.06	73.83 ± 3.45	44.23 ± 3.80	33.73 ± 1.11	77.76 ± 4.37	47.27 ± 3.01	47.59 ± 1.84
N	14	17	3	9	8	6	11
Sect.	8.95 ± 1.25			6.35 ± 0.77			9.62 ± 1.92
Vol.	2			2			2
Ell. Mod.	23.75 ± 0.55	38.66 ± 1.81	23.16 ± 1.99	17.66 ± 0.58	40.72 ± 2.29	24.75 ± 1.58	24.92 ± 0.96
Adj.	9.38 ± 0.55	16.59 ± 1.81	9.10 ± 1.99	6.44 ± 0.58	17.58 ± 2.29	9.87 ± 1.58	9.95 ± 0.96
Hypothalamus							
Vol.	27.59 ± 1.46	27.18 ± 1.54	27.97 ± 4.67	13.30 ± 1.04	40.60 ± 2.30	17.92 ± 1.31	20.70 ± 1.32
N	14	17	3	9	8	6	11
Ell. Mod.	14.45 ± 0.77	14.23 ± 0.80	14.65 ± 2.44	6.93 ± 0.54	21.26 ± 1.21	9.38 ± 0.69	10.64 ± 0.69
Dorsal medulla							
Vol.	14.37 ± 0.63	8.19 ± 0.36	12.08 ± 1.57	8.68 ± 0.37	19.89 ± 1.86	6.24 ± 0.23	2.41 ± 0.13
N	14	17	3	8	8	5	11
Ell. Mod.	7.52 ± 0.33	4.28 ± 0.19	6.32 ± 0.82	4.54 ± 0.20	11.30 ± 0.47	3.27 ± 0.12	1.26 ± 0.07
Olfactory bulb							
Vol.	0.967 ± 0.05	0.89 ± 0.05	1.00 ± 0.11	0.48 ± 0.03	1.34 ± 0.14	0.52 ± 0.06	0.43 ± 0.05
N	13	15	3	8	8	6	10
Ell. Mod.	0.51 ± 0.029	0.47 ± 0.028	0.53 ± 0.055	0.25 ± 0.017	0.70 ± 0.071	0.27 ± 029	0.24 ± .025

Total brain mass, raw volumes (gross brain measures and sectioned measures), and volumes obtained from the elliptical model (before and after adjusting from volumes obtained from sections) are provided. Sample sizes are noted.

[a] Volume measures reflect the raw LWH gross brain measures. [b] Sectioned volume measures. [c] The ellipsoid model for total brain volume only reflects the correction factor obtained from the regression of volume vs. mass, i.e., volume = LWH π (1/(6 × 1.23)). For all other structures, volume = LWH π (1/6). [d] Adjusted values reflect volumes of a given brain structure obtained from the elliptical model, using the correction factor obtained from sections. See methods for further details.

sures utilizing the ellipsoid model and volume measures obtained from sections (whole brain: $r^2 = 0.673$, $p < 0.03$; telencephalon: $r^2 = 0.759$, $p < 0.02$; optic tectum: $r^2 = 0.8427$, $p < 0.01$; cerebellum: $r^2 = 0.9224$, $p < 0.002$). These regressions were used to obtain a correction factor for the gross brain volumes obtained from the elliptical model (table 1). The slopes obtained from the regressions indicated that the ellipsoid model systematically overestimated the size of the whole brain, telencephalon, optic tectum and cerebellum by 24, 33, 107 and 33%, respectively. Some of the difference between gross brain and sectioned data is likely due to the estimated 25–30% shrinkage that occurs with the normal drying and dehydration process during staining. Note that the gross brain measure of the midbrain included the optic tectum plus other mid-brain structures (e.g., torus semicircularis, etc.), but the sectioned measure included only the optic tectum. Systematic errors like this, however, do not have any bearing on the statistical analyses conducted.

Allometry, Regression Residuals, and Brain Fractions

Structure sizes were compared with the rest of the brain (i.e., brain size minus the investigated area) to evaluate the extent to which variation in structure size could be explained by the rest of the brain size [Finlay and Darlington, 1995; Finlay et al., 2001]. Scaling relationships between biological variables can be described by the power function $Y = k X^{\alpha}$, where Y and X are the variables and k and α are the parameters [intercept and slope, respectively: Harvey, 1991; Barton and Harvey, 2000]. We conducted an analysis of covariance (ANCOVA) to estimate the interaction effect of brain structure and species which might arise from repeated sampling (i.e., several individuals per species). We found a marginally significant interaction term only for the midbrain ($F_{6,67} = 3.234$, $p = 0.054$), indicating that for this structure we cannot completely rule out that individual variability within species might affect the comparison across species.

Residuals for individual fish were calculated using the parameters α and k from the power regressions across all focal species according to the formula:

$$\text{Residual} = \text{structure size} / k \times (\text{brain size}^{\alpha})$$

A residual of 1 indicates that a structure size is predicted by this cross species fit. Residuals greater or less than 1 fall above or below the regression line; this indicates that the size of a brain structure in a given species is larger or smaller than expected, respectively.

Brain fractions were defined as the quotient of structure size with brain size [Clark et al., 2001]. For an individual brain, the sum of all brain fractions was <1, because the structures were not inclusive of the whole brain (e.g., the brainstem and ventral medulla were not measured). Analysis of variance (ANOVA) and the appropriate post-hoc tests (Tukey or Games-Howell) were used to compare residuals across species.

Exploring Relationships between Habitat Measures and Brain Structures

We calculated Pearson correlation coefficients to examine relationships between brain structures and socio-ecological variables. For each focal species, the average size of a particular brain structure (measured by both residuals and fractions) was compared with the average socio-ecological variable for that species. Because we did not have ecological data available for *X. bathyphila*, this species could not be included in this analysis. All statistical analyses were performed in SPSS v. 12.0 (SPSS Inc.). We report the original p-values as well as the (very conservative) significance threshold according to Bonferroni to correct for multiple hypothesis testing.

Independent Contrasts

Because of their common evolutionary history, traits across species within a hierarchical and branched phylogeny cannot be considered independent [Felsenstein, 1985; Pagel, 1999]. We used the CAIC software [Purvis and Rambaut, 1995; URL: http//www.bio.ic.ac.uk/evolve/software/caic/] to calculate phylogenetically independent contrasts [Felsenstein, 1985; Pagel, 1999] based on both the consensus tree and the maximum likelihood tree obtained from analysis of the variable part of the mitochondrial control region provided by Koblmüller et al. [2004]. Note that because the third tree these authors constructed (based on a combination of sequences from the cytochrome b and ND2 genes) did not contain all our focal species, we did not use this alternative tree. We set branch lengths to equal length [Garland et al., 1992] and found no correlation between the standardized independent contrasts versus their standard deviations for any variables analyzed in this study, indicating that the arbitrarily equalized branch lengths standardized the contrasts and were appropriate for our analysis [Diaz-Uriarte and Garland, 1996, 1998]. We used the 'CRUNCH' algorithm of the CAIC package for continuous variables; for the one dichotomous variable, social organization, we used the 'BRUNCH' algorithm [Purvis and Rambaut, 1995].

We calculated both linear regressions (forced through the origin) and Pearson correlations between standardized habitat and brain region contrasts obtained from either fractions or residuals based on either the consensus or maximum likelihood tree. For the dichotomous variable (social organization: monogamous vs. polygamous), t tests were conducted. Because calculating contrasts results in a decrease of degrees of freedom and thus smaller sample sizes (five contrasts for the consensus tree and four for the maximum likelihood tree), our statistical power to detect significant effects of habitat variables on brain structures was reduced compared to the original data. However, all four correlation analyses for standardized contrasts (for fractions or residuals based on either phylogeny) yielded similar trends. Therefore, we combined the p-values for the four separate tests conducted [see Sokal and Rohlf, 1994, page 795] to calculate a single p-value. Significant results are only expected if all four analyses yield similar results. If any of the comparisons yield highly non-significant or variable results, the resulting combined p value is unlikely to be significant. In other words, concordant and marginally significant results become more robust with this procedure, whereas spurious results become less likely.

Results

We present the data on quantitative habitat measures and neuroanatomical traits in cichlid species separately, and then examine the relationships between brain structures, the environment, and social organization.

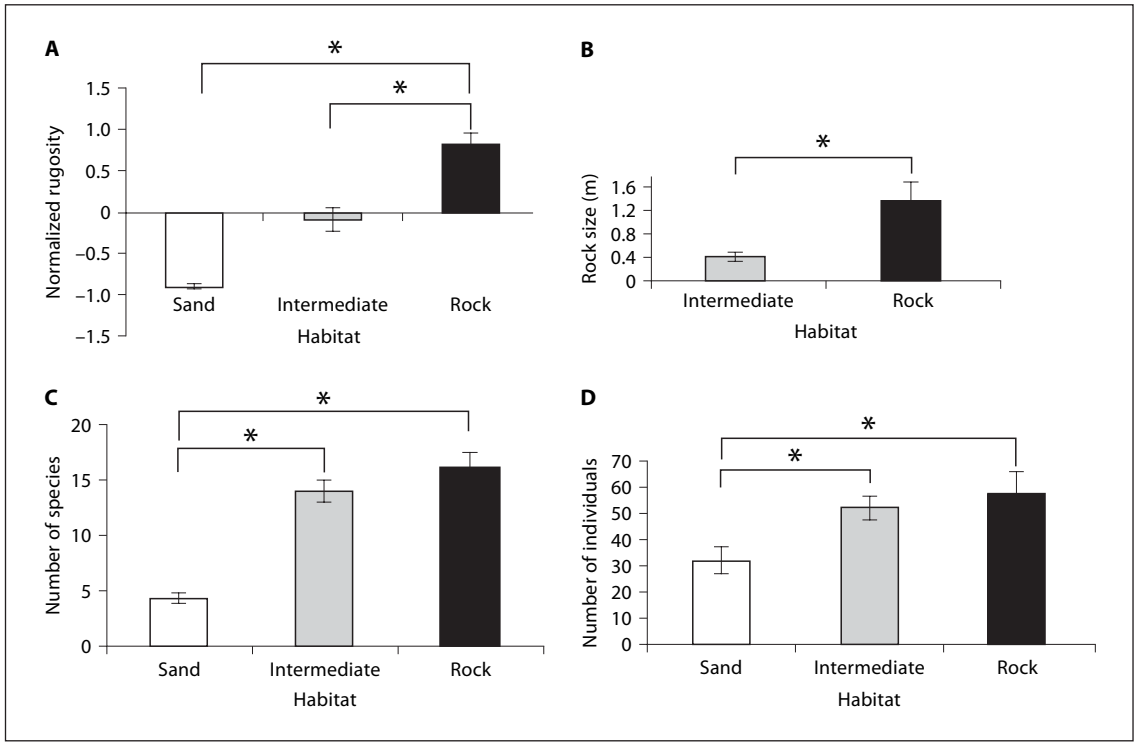

Fig. 3. Quantitative assessments (mean ± SE) of socio-ecological properties segregate qualitative habitat categories (sand: n = 11; intermediate: n = 17; rock: n = 10). **A** Comparison of normalized (z-transformed) rugosity measures across habitats. **B** Comparison of rock size for intermediate and rock habitats. No rocks were found in sandy habitats. **C** Comparison of species number across habitats. **D** Comparison of number of individuals across habitats. (* $p < 0.05$).

Ecology

Our measure of surface roughness (rugosity) provides a quantitative definition of habitat structure, which has traditionally been described by subjective, qualitative categories such as 'sand, intermediate, and rock' habitats. Average rugosity varied significantly for the three qualitative habitat types ($F_{2,35} = 35.446$; $p < 0.0001$). Average rugosity was significantly less in intermediate habitats compared with rock (fig. 3A; Games-Howell post-hoc test, $p < 0.001$) and smaller still in sandy habitats compared with rock ($p < 0.0001$). Average rock size was also significantly smaller in intermediate habitats compared to rock habitats (Student's $t = -2.932$; $p = 0.015$, fig. 3B). We never found any rocks of measurable size in sandy habitats. Light attenuation with depth also relates to habitat type (data not shown), likely a result of increased turbidity in sand habitats.

Social measures were significantly different across qualitative habitat categories (figs. 3C and D; number of species, $F_{2,35} = 36.102$, $p < 0.0001$; number of individuals across all species , $F_{2,35} = 4.789$, $p = 0.015$). Sandy habitats contained significantly fewer species (Tukey post-hoc tests, $p < 0.0001$) and individual fish ($p < 0.05$) compared with both intermediate and rocky habitats (n.s., $p = 0.236$).

Physical and social measures were highly correlated. As shown in table 2, both the number of species and the number of individuals (across all species) found in any given quadrat were strongly positively correlated with increased structural complexity of the habitat. The association between number of species and number of adult conspecifics (averaged across the six focal species for which data were available) is marginally significant ($p = 0.061$).

By averaging habitat measures across quadrats in which a given focal species occurred, significant differences were found among the focal species (table 3). Rugosity, rock size, and the number of species varied widely in the habitats of the six focal species (fig. 4). For example, rugosity was significantly smaller where *X. flavipinnis*

Table 2. Correlations (Pearson's r, with significance p) of physical and social variables

Habitat measure	Rugosity	Rock size	Species #	Individual fish #
Rock size				
r	0.521	–	–	–
p	0.001*			
Species #				
r	0.662	0.472	–	–
p	<0.0001*	0.003*		
Individual fish #				
r	0.356	0.289	0.550	–
p	0.028*	0.083	<0.0001*	
Conspecifics #				
r	–0.681	–0.610	–0.791	–0.190
p	0.136	0.198	0.061	0.718

Significant relationships are noted by an asterisk. The number of species and the number of individual fish per quadrat are positively correlated with habitat complexity.

Table 3. Comparison of several physical and social habitat measures across focal species, as judged by ANOVA

Measure	d.f.	F	p
Rugosity	5,48	8.106	<0.0001*
Rock size	5,46	5.185	0.001*
Species #	5,48	7.567	<0.0001*
Individual fish #	5,48	2.398	0.051
Conspecifics #	5,47	2.176	0.073

Significance is noted by an asterisk. Habitat complexity and numbers of species showed significant variation across the six Ectodini species examined. d.f. = Degrees of freedom.

Table 4. Power and linear regressions of brain structure sizes against the rest of the brain size, and brain size against body size

Structure	d.f.	Power regression		Linear regression	
		exponent	R^2	slope	R^2
Telencephalon	66	0.755	0.624	0.167	0.642
Olfactory bulb	62	1.005	0.502	0.004	0.512
Dorsal medulla	**64**	**0.928**	**0.180**	**0.057**	**0.323**
Hypothalamus	67	1.042	0.761	0.156	0.759
Midbrain	68	0.803	0.711	0.413	0.641
Cerebellum	65	1.037	0.660	1.036	0.685
Whole brain	64	0.461	0.608	n/a	n/a

d.f. = Degrees of freedom. For all regression coefficients, $p < 0.001$ except for the linear regression of the dorsal medulla, $p = 0.01$. Overall brain size explained over half of the variation for all structures, with the exception of the dorsal medulla (highlighted in bold).

was found, compared with habitats preferred by *A. leptura* (Tukey post-hoc, $p < 0.0001$). The focal species did not differ in number of individual fish/quadrat. Group size was not measured, but is known to range from two (for adults from the monogamous species) to 10–60 (for adults from the polygamous species, depending on sex and reproductive activity) [Konings, 1996].

Neuroanatomy

We performed regression analyses for the absolute size of each brain structure against the size of the rest of the brain using both a power relationship and a linear relationship (table 4). All coefficients for power and linear regressions were highly significant (all at the level of $p < 0.001$, except for the linear regression of the dorsal medulla: $p < 0.01$). Overall brain size explained 50–76% of the variation for all structures, with the exception of the dorsal medulla (highlighted in bold). For that structure, brain size explains even less of the variance (18–32%).

To examine the data without making the assumption that brain structures scale linearly, residuals were calculated from power relationships, and analysis of variance (ANOVA) was used to compare average residuals across species. The residuals of all structures divided into multiple discrete homogeneous subsets, with each subset including species that do not differ significantly according to the Tukey post-hoc test, and the different subsets indicating groups that do (fig. 5A through F). The multiple subsets indicate a considerable amount of evolutionary variation within this group of closely related species. Note that there appears to be a relationship between residual size of a brain structure and the preferred habitat and/or social organization of a species (see below).

ANOVA was similarly conducted on the fraction of each structure with respect to the whole brain (table 5). All structures divided into discrete homogeneous subsets at the $p < 0.05$ level (data not shown). The results were in line with those for residuals described above. Importantly, the telencephalic fractions for all four monogamous species were larger than those of the polygamous species.

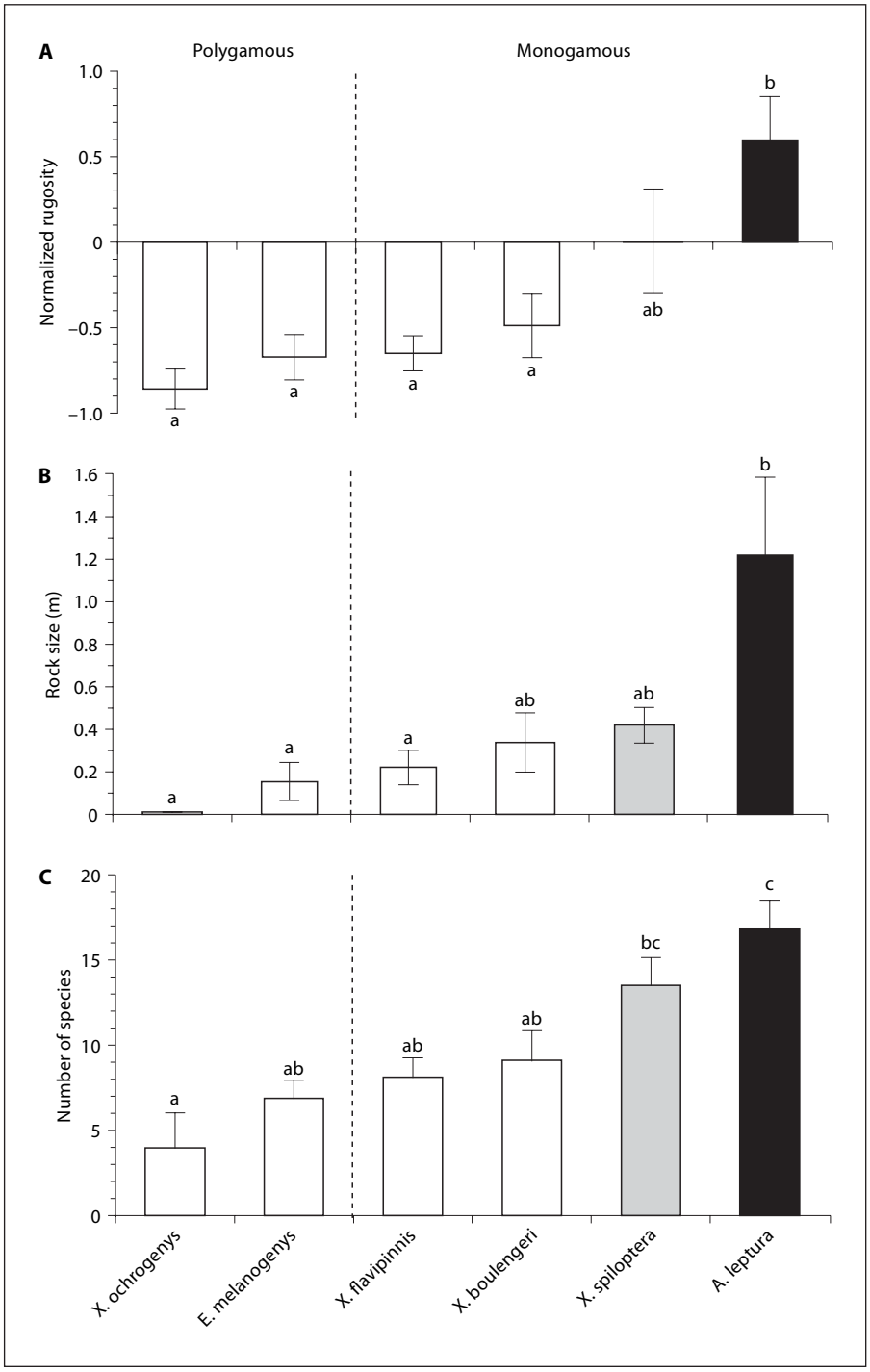

Fig. 4. Quantitative assessments (mean ± SE) of physical habitat properties vary significantly across the six focal Ectodini species for which ecological data was obtained. **A** Rugosity, **B** rock size and **C** species number. All measures were taken in 5 × 5 m quadrats (n = 38). The bars indicate preferred habitats (sand (white); intermediate (gray); rock (black)). Polygamous species are to the left of the hatched vertical line, monogamous species to the right. Letters denote homogeneous subsets at $p < 0.05$, as determined by ANOVA followed by Tukey post-hoc tests.

Relationships between Neuroanatomy and Socio-Ecological Measures

We constructed covariance matrices for brain structures vs. habitat properties by calculating Pearson correlation coefficients for both fractions and residuals. Note that because we did not have quantitative ecological data available for *X. bathyphila*, we performed these analyses on six species. Because Social Organization is a dichotomous variable (monogamous vs. polygamous), t tests were used instead of correlation. Figure 6A, B shows the

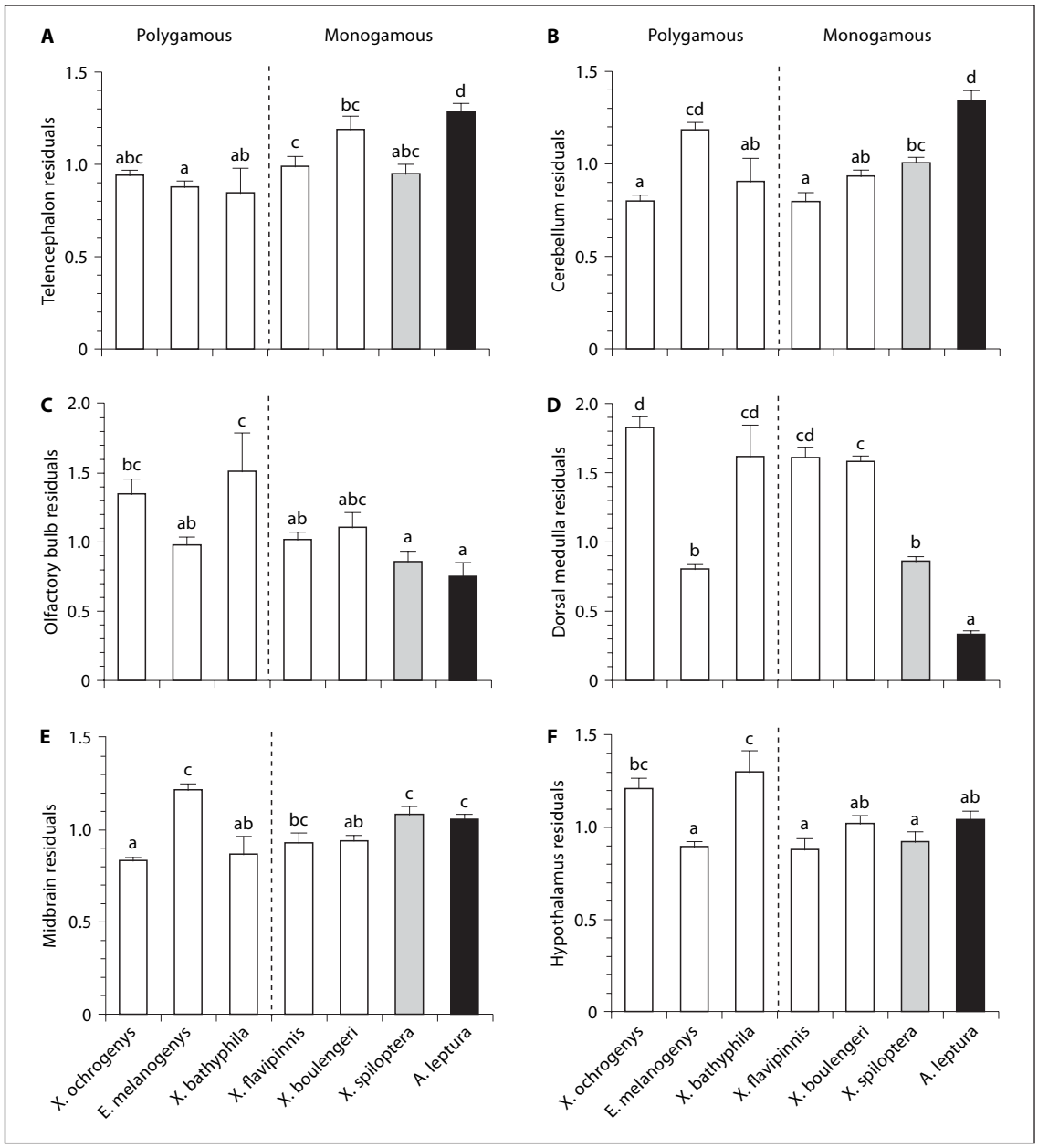

Fig. 5. Residual measures show that some brain areas vary significantly across species relative to socio-ecological variables. Residuals (mean ± SE) are shown for **A** telencephalon, **B** cerebellum, **C** olfactory bulb **D** dorsal medulla, **E** midbrain and **F** hypothalamus. The bars indicate preferred habitats (sand (white); intermediate (gray); rock (black)). Polygamous species are to the left of the hatched vertical line, monogamous species to the right. Letters denote homogeneous subsets at $p < 0.05$, as determined by ANOVA followed by Tukey post-hoc tests.

two color-coded matrices, where the color of each cell is determined by the strength and direction of the respective association (red: positive; blue: negative). Significant results are indicated by symbols ($^{+}p < 0.10$; * $p < 0.05$; ** $p < 0.01$). Given that we had only six species available, it is not surprising that none of the p-values reaches the very conservative Bonferroni-corrected significance threshold ($p = 0.0012$). However, the results for fractions and residuals are highly concordant, which supports the validity of the observed patterns.

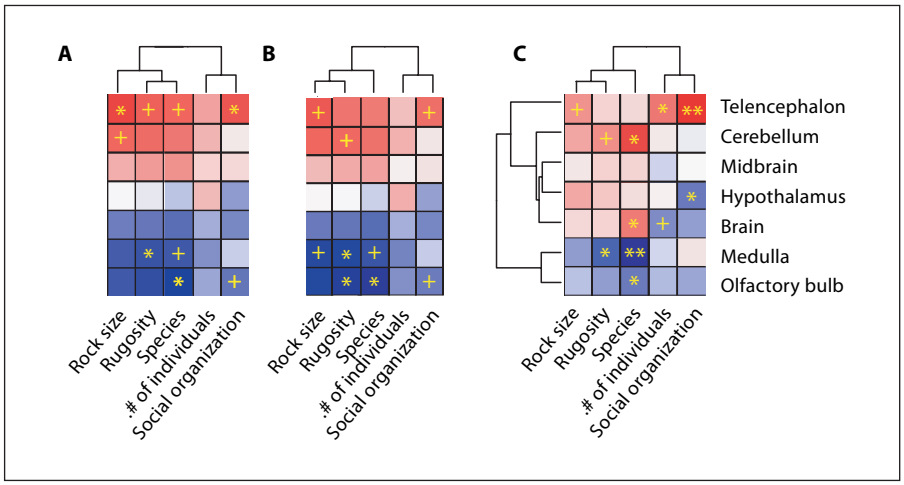

Fig. 6. Color-coded covariance matrices showing the statistical strength and the direction of the associations between habitat and social variables on one hand and neural measures on the other. Results are shown for **A** fractions, **B** residuals and **C** independent contrasts [results combined from fraction- and residual-based contrasts using both the consensus and maximum likelihood trees presented in Koblmüller et al., 2004] (table 6). **C** The cladograms show the results of hierarchical clustering along both axes. The color gradient runs from dark blue (strong negative association: $p < 0.01$) to dark red (strong positive association: $p < 0.01$), with light colors indicating weaker relationships. Significant associations are indicated (* $p < 0.05$; ** $p < 0.01$), as are trends ($^{+}p < 0.10$). For continuous habitat and social variables, the results are based on Pearson correlation coefficients. For Social Organization (the only dichotomous variable), the results are based on t-tests: red indicates that a brain region is larger in monogamous species, and blue that it is larger in polygamous species. Several environmental variables strongly affect numerous brain structures in Ectodine cichlids. Generally, the telencephalon and cerebellum increase as environmental complexity increases, whereas the olfactory bulb and the dorsal medulla decrease. Social organization selectively affects the telencephalon and hypothalamus. The telencephalon is larger and the hypothalamus smaller in monogamous species.

Table 5. Comparison of variability in the size of different brain structures across focal species, as judged by ANOVA

Brain structure	d.f.	F	p
Telencephalon	6,60	18.467	<0.0001
Olfactory bulb	6,56	6.432	<0.0001
Dorsal medulla	6,58	108.547	<0.0001
Hypothalamus	6,61	10.253	<0.0001
Midbrain	6,61	17.570	<0.0001
Cerebellum	6,59	30.401	<0.0001

Data shown are for fractions. All structures showed highly significant variation across the seven Ectodini species examined. d.f. = Degrees of freedom.

In order to correct for the potentially distorting phylogenetic effects, we then calculated the standardized independent contrasts for all measures (for habitat as well as brain structure fractions and residuals) using both the consensus and maximum likelihood trees established by Koblmüller et al. [2004]. Because all four sets of analyses gave similar results, we calculated a single χ^2-value/p-value for each comparison between habitat variable and brain structure (table 6; see Methods).

The overall pattern of the covariance matrix obtained from independent contrasts is very similar to the results obtained from the original fractions and residuals, with the exception of the hypothalamus and total brain size (fig. 6C). Measures of habitat complexity (such as the direct measures of rugosity and rock size, and the indirect measure of species number, which is strongly correlated with habitat complexity) affect cichlid brains in a similar fashion. Specifically, increased habitat complexity is significantly associated with larger brains and cerebella; the telencephalon showed a trend. Conversely, the olfactory bulb and dorsal medulla showed strong negative correlations with habitat complexity. The size of the midbrain (which includes the optic tectum) and hypothalamus appeared habitat-invariant. The fact that we find such strong associations with only six species is very encouraging, as it hints at a strong influence of habitat complexity on brain structure in this clade.

Table 6. The size of brain structures varied with physical and social properties for Ectodine cichlids

	Whole brain	Telencephalon	Cerebellum	Midbrain	Hypothalamus	Dorsal medulla	Olfactory bulb
Rugosity	5.103	5.152	*14.279*$^{+}$	5.321	6.708	**19.128***	11.596
Rock size	4.495	*13.579*$^{+}$	11.006	2.693	10.717	11.810	6.872
Species #	**16.834***	4.416	**22.452***	4.990	3.871	**26.568****	**16.174***
Individual fish #	*13.866*$^{+}$	**17.436***	2.436	4.875	1.490	4.476	7.756
Conspecifics #	4.890	2.370	11.133	8.609	10.812	7.536	*14.015*$^{+}$
Social organization	11.472	**24.273****	1.908	0.673	**16.262***	3.068	10.422

The table shows the χ^2 values that result from combining the four p values obtained from standardized independent contrasts for fractions and residuals based on both the consensus and maximum likelihood trees presented by Koblmüller et al. [2004]. Significant associations are bold (* $p < 0.05$; ** $p < 0.01$); trends are italic ($^{+}$ $p < 0.10$). Since adjusting for multiple hypothesis testing is very conservative ($p_{Bonferroni} = 0.0012$), unadjusted p-values are reported to allow for a more complete evaluation of the data.

Social measures (such as social organization, number of individual fish and number of adult conspecifics) also influence brain structure. The strongest relationship is between social organization and telencephalic size, with the telencephalon consistently larger in the monogamous species. Figure 7 maps the relationship between social organization and telencephalic size onto the phylogeny: the fraction of the brain occupied by the telencephalon was significantly different between the four monogamous/biparental (16.13 ± 1.32%) and three polygamous/maternal (12.94 ± 0.47%) species examined ($t_5 = -2.71$, $p = 0.045$). A similar result was obtained with residuals (as indicated in fig. 6B). Interestingly, polygamous species have a larger hypothalamus than monogamous species, although this relationship is not influenced by the number of heterospecific or conspecific fish in the habitat. Whether the trend towards a positive association between the number of conspecifics and the size of the olfactory bulb is real remains to be seen.

Overall, the pattern obtained with social measures is quite different from that seen with habitat variables. Five out of seven of the brain measures show a relationship with habitat measures. In contrast, only two brain structures, the telencephalon and hypothalamus, are correlated with social factors. The hierarchical cluster analysis in figure 6C shows a close relationship between species number and physical habitat measures on the one hand, and social organization and number of individuals on the other. Dorsal medulla and olfactory bulb group together, as do midbrain and cerebellum. Interestingly, telencephalic size emerges as outgroup in this analysis.

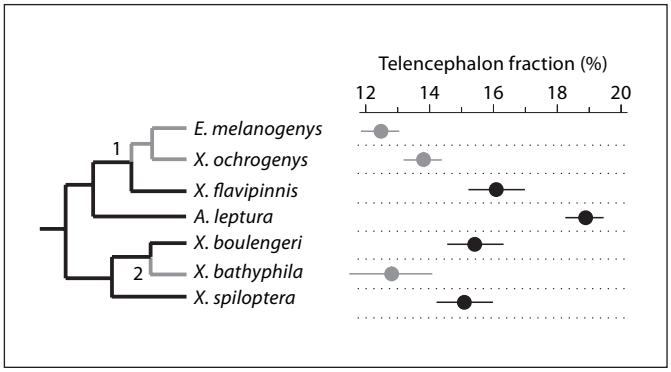

Fig. 7. Comparison of telencephalic size (fractions) as a function of social organization for three monogamous (black) and four polygamous (gray) species mapped onto a phylogenetic tree within the Ectodini clade. Note the repeated, likely independent, transitions from monogamy to polygamy in this clade [two out of four are shown here; see also Koblmüller et al., 2004]. Wherever polygamy occurs, the telencephalon is smaller ($p = 0.0137$, as judged by independent contrasts). Tree based on Koblmüller et al. [2004].

Discussion

In the present study, we asked how much of the variation in the size of major brain regions in a monophyletic clade of Tanganyikan cichlids could be explained by social and ecological variables. We established quantitative measures for habitat complexity, and we showed that quantitative measures of the physical and social habitat are highly correlated. We confirmed, at a finer level of analysis, the results of van Staaden et al. [1995] and Huber

et al. [1997] showing that the size of major brain structures may have been under selection during cichlid evolution. Our results, together with theirs, show that structure size is highly variable across species. Most importantly, we found that environmental and social factors differentially affect the brain, with environmental factors showing a much broader effect on a range of brain structures compared to social factors. Our data set of closely-related species provides new insights as to whether and how physical or social factors have sculpted brain and behavior throughout evolution.

Ecology

Properties of the social and physical environment are linked in near-shore benthic habitats of Lake Tanganyika. We surveyed 38 quadrats, inhabited by 65 species of fish (including 61 cichlid species). We found that habitat structure (rugosity and rock size) is positively correlated with the number of species and the number of individuals present. This finding is new for freshwater fish communities and consistent with studies linking species richness to rugosity in coral reef communities [Luckhurst and Luckhurst, 1978].

Potential confounds must be recognized in treating measures of socio-ecology as explanatory variables. Habitat properties not measured in the present study that might be expected to affect neural structure include diet complexity, length of foraging route and manipulation of the environment (e.g., bower building in cichlids) [Barlow, 2000; Madden, 2001; Bshary et al., 2002]. Social properties not measured that could be relevant explanatory variables include social learning, social dominance structure, competition for access to mates, and group size, which depends on age, sex, and reproductive activity [Byrne and Whiten, 1988; Konings, 1996; Bshary et al., 2002; personal observation].

Neuroanatomy

We determined the relationship between brain structure sizes and total brain sizes in order to identify areas that might be under selective pressure. We chose to adopt the quantitative approach used by van Staaden et al. [1995], Huber et al. [1997], Wagner [2001a, b] Lisney and Collin [2006] and Lisney et al. [2007] to estimate the size of brain structures from length, width, and height measures of intact brains. We used estimates from multiple samples of each species to prevent individual variations from affecting conclusions about each species. The ellipsoid model we and the previous authors used might not account for certain shape changes such as changes in a concave portion of a structure (as with the telencephalon), or the crescent shape of the optic tectum. Such a model might also be problematic if there were significant inter-species variations in shape of various structures. In our system, however, we compared only closely-related species, where drastic shape changes did not occur. Further, to validate the gross brain measures, we sectioned the brains of three species. We found that the ellipsoid model, although overestimating volumes of the brain, telencephalon, and cerebellum by 24–33%, nevertheless provided consistent estimates of brain area volumes within the Ectodini clade. The 107% overestimate of the optic tectum was likely due to the fact that the gross brain measure included other midbrain structures, whereas the sectioned measure was optic tectum only.

Although the size of the brain explained more than half of the variation in all structures except the dorsal medulla, indicating developmental constraints, we also found considerable variation that could not be predicted by the size of the rest of the brain (table 4). Measures of the dorsal medulla showed the most unexplained variation. Interestingly, the size of the brain could explain only 62% of the variation in telencephalon size. In other words, 38% of the remaining variation is not accounted for by developmental constraints. Thus natural and/or sexual selection or drift likely have acted on the cichlid telencephalon in a mosaic fashion. This result is supported by the cluster analysis which separates the telencephalon from all other structures (fig. 6). The considerable variation in cichlid telencephalic size with respect to the brain is consistent with earlier work on teleosts [Ridet and Bauchot, 1990; van Staaden et al., 1995; Huber et al., 1997; Kotrschal et al., 1998]. In birds, only 20% of the remaining variation appears to be unexplained by brain size [Burish et al., 2004].

We used both residuals and brain fractions to compare the size of brain structures in different species and to explore relationships between structure size and properties of the environment. Both methods of comparison provided evidence for mosaic evolution and suggested similar relationships with properties of the environment. We used residuals from power regressions to compare structure sizes because such analysis does not assume that structures scale linearly with the rest of the brain. However, because the value of residuals can change depending on the set of samples used, we also used brain fractions, which are a property of each sample. We found that linear regressions explained as much variation as power regressions (table 4), justifying the comparison of brain fractions from brains of different sizes.

By conducting a parallel analysis using standardized independent contrasts, we were able to isolate the role of physical and social factors in cichlid brain evolution independent of phylogenetic history. Overall, these results were concordant with those obtained for the original data (fractions and residuals; fig. 6). Although the fine branch points of the phylogeny are uncertain, our results were consistent for both fractions and residuals when we analyzed the data according to the consensus tree or an alternative phylogeny based on a maximum likelihood tree obtained from the mitochondrial control region [Koblmüller et al., 2004]. These results show that environmental and social factors differentially affect the brain, with environmental factors showing a much broader effect on a range of brain structures compared to social factors. Five out of seven of the brain measures show a relationship with habitat measures. Brain size and cerebellum are positively associated with species number, which is correlated with habitat complexity, as noted previously; the medulla and olfactory bulb are negatively correlated with habitat measures. The telencephalon shows a trend toward a positive correlation with rock size. In contrast, only two brain structures, the telencephalon and hypothalamus, are strongly associated with social factors. Specifically, social organization and individual number are positively correlated with telencephalic size; social organization is negatively correlated with hypothalamic size.

Cluster analysis of the independent contrasts (fig. 6C) illuminates the evolutionary relationship among brain structures. The close relationship between species number and habitat measures on the one hand, and social organization and number of individuals on the other suggest that they have corresponding effects on brain structure. Dorsal medulla and olfactory bulb, two structures that are involved in foraging among other functions, appear to coevolve under the influence of the same habitat variables. Similarly, midbrain and cerebellum cluster together, suggesting coevolution of these multimodal structures involved in spatial processing. Finally, a unique combination of selection pressures appears to influence telencephalon size.

Relationships between Neuroanatomy, Habitat Structure, and Social Organization

Comparing brain structure sizes with socio-ecological measures can help reveal adaptive pressures. However, identification of the environmental and social forces shaping brain evolution is confounded by several factors. First, properties of the physical and social environment often correlate [Luckhurst and Luckhurst, 1978; Barton, 1996]. In our study, we have shown that rugosity, rock size, species number and individual number all co-vary, making it difficult to isolate the salient selection pressure, if indeed there is just one. Second, a given cognitive skill might confer advantages in both social and physical spheres [Reader and Laland, 2002; Bshary et al., 2002]. Therefore, selection pressures related to both the physical and social environment could simultaneously have affected the same neural phenotypes [Barton and Dunbar, 1997].

Higher-Order Structures

Previous studies directly implicated the fish telencephalon in several complex behaviors such as spatial learning [Salas et al., 1996a, b, 2003; Lopez et al., 2000], fear conditioning [e.g., Portavella et al., 2003, 2004], learning, territoriality, courtship, spawning, parenting, and schooling [review: Demski and Beaver, 2001; Shinozuka and Watanabe, 2004]. In this study, we have shown that the size of the cichlid telencephalon within the Ectodini clade positively correlates with habitat measures; this confirms, at a finer scale, the result from Huber et al. [1997]. We recently showed that cichlid visual acuity is also positively correlated with rugosity [Dobberfuhl et al., 2005]. We hypothesize that the demands for higher-order processing of sensory information, as well as cognitive abilities, led to increased telencephalon size in animals from more highly structured habitats, particularly in highly visual animals such as cichlids. Note that Bauchot et al. [1977] also found large brains and larger forebrains for mobile reef fishes living within complex reef structures, compared to more sedentary species, although the ecological differences were not quantified.

A larger forebrain might also provide the additional computational capacity necessary for more sophisticated navigation and learning to find (and return to) shelter, food, and mates in a complex three-dimensional environment. Although homologizing fish and mammalian forebrain structures is difficult, multiple lines of evidence imply that certain telencephalic structures and their functions are highly conserved. In particular, immunocytochemical, developmental, and connectional studies suggest that teleost areas Dl and Dm are homologous to the mammalian hippocampus and amygdala, respectively [Northcutt, 1995; Wullimann and Mueller, 2004]. Ablation studies have demonstrated the importance of Dl in spatial learning and Dm in emotional (fear) learning [Portavella et al., 2002, 2004; Salas et al., 2003]. Both areas receive multimodal sensory input, including visual input [Northcutt, 2006].

Because habitat complexity is correlated with increased numbers of both species and individuals, the frequency of interactions between individuals is expected to increase as well. This increased social interaction might also have led to an expansion of cognitive abilities, and our results do indeed show a significant positive correlation between numbers of individual fish per quadrat and telencephalon size. The only way to separate the effects of habitat and social influences is to test their role separately in developmental plasticity experiments. Such experiments are currently underway.

One of the most exciting outcomes of this study is the significant relationship between telencephalon size and social organization (fig. 6, 7). We have shown that telencephalic size can be 15–20% larger in monogamous species compared to sister, polygamous species. The seven species that we studied included two transitions between monogamy and polygamy of the four transitions predicted by a consensus phylogeny of the Ectodini clade [Koblmüller et al., 2004]. Our work is the first study showing this relationship in a monophyletic clade of closely-related species. We do not know of any corresponding work across primate or bird species that has shown significant differences of brain structure size relative to mating systems, although differences in neuropeptide systems [Young et al., 2005] and sexual dimorphism in hippocampal size [Jacobs et al., 1990] have been shown between monogamous and polygamous voles. The effect of social organization on cichlid telencephalic expansion is consistent with existing hypotheses of social influences on telencephalon evolution [primates: Milton, 1988; Barton and Dunbar, 1997; birds: Burish et al., 2004; Goodson et al., 2005, but see Beauchamp and Fernandez-Juricic, 2004 for an opposing view].

The functional consequences of the forebrain difference between monogamous and polygamous cichlid species are unclear, but might relate to (individual) mate recognition and/or paternal care for the offspring. But, because mating preference and parental care go hand in hand in cichlids (e.g., monogamous species are always biparental), determining whether telencephalic expansion is a result of mating preference, parental care, or both is difficult. One possible way to explore this question might be to exploit the remarkable neural and behavioral plasticity for which cichlids are known [Barlow, 2000; Hofmann, 2003]. For example, one could potentially experimentally manipulate the social environment to produce a particular mating type or parenting outcome [e.g., manipulation of parental care in birds; Stoleson and Beissinger, 1997].

What happens when a highly structured habitat and monogamy co-occur in a species? We found that the rock-dwelling, monogamous *A. leptura* had the largest telencephalon of all species examined (32% larger than the polygamous species; see fig. 7). We intend to compare this result to a second rock-dwelling monogamous species *(Xenotilapia papilio)* to see if our prediction of a greatly expanded telencephalon will hold true. It is important to note, however, that we have shown that habitat complexity is confounded with certain other social measures, specifically species number and individual number.

Another interesting result is that the hypothalamus appears enlarged in males of polygamous species. These males form lek-like structures [Barlow, 2000], in which they vigorously display dominance and territorial behaviors as well as courtship and mating behavior. These behaviors do occur in monogamous species, but less frequently and with less intensity [unpublished observations]. As the hypothalamus controls many of the neuroendocrine pathways underlying these behaviors, this difference in social organization may well have favored morphological adaptations that resulted in an increased hypothalamic volume. Interestingly, studies in another lekking Tanganyikan cichlid, *Astatotilapia burtoni,* show that certain hypothalamic cell types, which produce neuropeptides such as gonadotropin-releasing hormone or somatostatin, are enlarged in territorial and reproductively mature individuals [Francis et al., 1993; Hofmann and Fernald, 2000]. Although these parallels are intriguing, whether such differences can affect the volume of the entire hypothalamus has not yet been determined.

Finally, the positive correlation between cerebellum and habitat complexity could relate to increased sensorimotor integration and motor coordination in three-dimensional physical environments, but samples from additional species are required for such an argument. Day et al. [2005] recently demonstrated expansion in cerebellar size with increased bower complexity among bower birds. Evolutionary variation in cerebellar size correlated with expansion in the telencephalon has been shown in primates [MacLeod et al., 2003].

Sensory Areas

It may seem surprising that the size of the optic tectum/midbrain, both a primary sensory visual structure and a multimodal sensory structure, is independent of habitat complexity. Complex three-dimensional structures likely pose challenges for visual behaviors related to navigation, mate finding, and predator avoidance; thus

an increase in tectal size could allow for faster and/or more sophisticated visual processing and memory. For example, T. Lisney and S. Collin [personal communication] have recently found larger optic tecta in reef-associated sharks compared to benthic species. Of course, habitat (or social) structure might not be the only factor shaping tectal evolution. The tectum, like the cerebellum, plays a role in sensorimotor integration. Huber et al. [1997] found in East African cichlids that piscivorous species and others that utilize motile prey have a larger tectum. These authors suggested a relationship between tectum size and feeding specialization that we cannot explore here, as all species examined have very similar trophic modes (benthic feeders).

The size of both the olfactory bulb and the dorsal medulla is negatively correlated with habitat measures (rugosity and species number), as it is larger in the sand-dwelling, zooplanktivorous fishes. Previous studies have linked fish dorsal medulla size to feeding type [Ridet and Bauchot, 1990; Huber et al., 1997], which is consistent with its role in the processing of taste information. Sifting the sand for food, typical for the sand-dwellers studied here, is potentially a demanding gustatory and olfactory task. We therefore suggest that the correlations for dorsal medulla and olfactory bulb are likely confounded by the more direct pressure of feeding type on the structure and not so much a result of low rugosity or species number.

Next Steps

The brain structures measured in this study comprise functional regions over which selection pressures could have differed during evolutionary time. Unless structure is linked to function, however, interpretation of structural variation will remain subject to the criticisms brought forward against adaptationist 'just-so-stories' [Gould and Lewontin, 1979]. We have therefore begun behavioral, molecular, and plasticity studies to better link structure and function. First, we recently showed that visual acuity differs with respect to habitat preference and social organization [Dobberfuhl et al., 2005] and are currently exploring spatial memory differences across species. Second, a custom-made cichlid cDNA microarray enables us to take a more functional approach by identifying genes that are likely involved in certain brain areas in development and/or expression of the behavioral differences observed between species [Renn et al., 2004]. Finally, in an effort to separate the confounding effects of habitat complexity and certain social measures (numbers of species and individuals), we have begun to assess the amount of neural plasticity in developing animals.

Conclusion

Cichlid fishes are characterized by their rapid evolution, diverse habitat preferences, and an extraordinary range of social behaviors. In this study, we have shown a correspondingly large degree of variation in brain structures among closely-related species. The fact that the neural variation is correlated with differences in habitat preference and social organization strongly suggests that adaptive evolution has acted on cichlid brains. These factors appear to influence the brain differently. Habitat factors exert a broader effect on brain structure than social ones; social factors appear to influence primarily the telencephalon. The diverse physical and social environments across closely related species of cichlids highlight the advantages of this model system to studying adaptations in neural phenotype.

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