Behavioural Brain Research 221 (2011) 189–196

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Variation in asymmetry of the habenular nucleus correlates with behavioural asymmetry in a cichlid fish

Cristian Gutiérrez-Ibáñez [b,1], Adam R. Reddon [a,1,2], Michael B. Kreuzer [b], Douglas R. Wylie [a,b], Peter L. Hurd [a,b,*]

[a] Department of Psychology, University of Alberta, Edmonton, AB T6G 2E9, Canada
[b] University Centre for Neuroscience, University of Alberta, Edmonton, AB T6G 2E9, Canada

ARTICLE INFO

Article history:
Received 29 October 2010
Received in revised form 23 February 2011
Accepted 1 March 2011

Keywords:
Cerebral lateralization
Neural asymmetries
Habenula
Amatitlania nigrofasciata
Convict cichlid

ABSTRACT

Asymmetries in brain and behaviour have been demonstrated in numerous species representing all major vertebrate taxa, and may be a universal feature of the vertebrate nervous system. While descriptions of lateralization at the behavioural and neuroanatomical level are widespread, examples of correlation between asymmetries in behaviour and neural structures remain relatively scarce. In the past few years, the habenular nucleus has emerged as a potential site for the neural basis of some lateralized behaviours. Here we investigate the relation between continuous individual variation in asymmetry of the habenulae and behaviour in the detour task in the convict cichlid (*Amatitlania nigrofasciata*). We found that both male and female convicts show a significant population-level bias towards relatively larger left habenulae. We also show that habenular asymmetry is correlated with behavioural lateralization in both males and females, but in opposite directions. This adds to previous studies showing both in convict cichlids and other vertebrates an interaction between sex and lateralized behaviour. The results of this study increase our understanding of the role of the habenula in lateralized behaviour and highlight the importance of a comparative approach to understanding the development and evolution of habenular asymmetry.

© 2011 Published by Elsevier B.V.

1. Introduction

Asymmetries in brain and behaviour have been demonstrated in numerous species representing all major vertebrate taxa, and may be a universal feature of the vertebrate nervous system [1–3]. The ubiquity of behavioural asymmetries among all vertebrates studied so far suggests that it is an evolutionarily ancient adaptation that may have important adaptive functions [4–6]. In support of this hypothesis, it has been shown that more strongly lateralized animals possess cognitive advantages [7], for example, lateralized animals may be better able to attend to multiple stimuli simultaneously [8–10].

Understanding the neural underpinnings of lateralized behaviour represents a major frontier for comparative lateralization research. While descriptions of lateralization at the behavioural level are widespread, the examples of correlation between behavioural lateralization and neural structure remain relatively scarce (but see [11] for recent examples). A noteworthy exception is found in the anatomy of the visual system in birds. In chicks and pigeons, differential exposure to light of one eye during development leads to an anatomical asymmetry in the visual pathway, and this neural asymmetry is related to at least some lateralized behaviours [12–14].

In the past few years, the habenular nucleus has emerged as a potential site for the neural basis of some lateralized behaviours. This paired structure is a component of the epithalamus, and lies in the dorsal part of the diencephalon of all vertebrates [15]. The habenula is part of a highly conserved pathway (the dorsal diencephalic conduction system; [16]) that connects limbic regions of the forebrain with motor circuits of the brainstem (reviewed in [15–17]). The habenula appears to play a role in a diverse array of functions including pain perception, olfaction, feeding, mating and stress responses [17–19]. In mammals, the habenula is involved with the motivational control of behaviour [17,20]. In particular the habenula appears to be activated when the outcome of behaviour is aversive or is less-rewarding than was expected [17,20]. Activation of the habenula inhibits dopaminergic reward systems downstream, and may act to inhibit responding to non-rewarding stimuli [21]. Recent work suggests homology

---

* Corresponding author at: Biological Sciences Building, Room p-217, University of Alberta, T6G 2E9 Edmonton, Alberta, Canada. Tel.: +1 780 492 3578; fax: +1 780 492 1768.

E-mail addresses: cagutier@ualberta.ca (C. Gutiérrez-Ibáñez), reddonar@mcmaster.ca (A.R. Reddon), kreuzer@ualberta.ca (M.B. Kreuzer), dwylie@ualberta.ca (D.R. Wylie), phurd@ualberta.ca (P.L. Hurd).

[1] These authors contributed equally to this work and should be jointly considered first author.

[2] Current address: Department of Psychology, Neuroscience and Behaviour, McMaster University, Hamilton, Ontario, Canada L8S 4L8.

0166-4328/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.bbr.2011.03.001

of structure and function between the piscine and mammalian habenulae [22].

One major characteristic of the habenular nucleus is the marked neuroanatomical and/or neurochemical asymmetries seen across all major groups of vertebrates (reviewed in [23]). Among fish, reptiles and amphibians habenular asymmetry tends to be pronounced and consistent in its' direction within groups, but variable between them. For example, in lampreys and hagfishes the right habenula is markedly larger than the left, while in chondrichthyes the opposite is true [24–26]. A clear exception among the fish are the teleosts, in which the habenula has been described mostly as bilaterally symmetric, and only a few species have been reported to present clear anatomical asymmetries [23]. In contrast to the constancy of direction found in other groups, the teleost species that do present habenular asymmetries exhibit both leftward and rightward biases [23].

Zebrafish (*Danio rerio*) have served as a model for the study of both the development of neuroanatomical lateralization in vertebrates [27] and, more recently, the relation between brain asymmetry and behavioural lateralization [28–30]. Wild-type zebra fish typically have larger left habenulae [31], and several authors have found concordance between the direction of lateralization of the habenula and behavioural lateralization ([28,32,33] but see [34] for negative results).

These studies make use of the *frequent situs inversus* (*fsi*) strain of zebrafish, which results in an unusually high number of individuals with completely reversed neural and bodily asymmetries including the asymmetry of the habenular nucleus (resulting in rightward rather than leftward biased habenulae). All of the aforementioned studies treat habenular asymmetry as a dichotomous left-bias vs. right-bias trait and test for associations between the direction of this asymmetry and the behaviour of the individual.

Recently Reddon et al. [35] showed that in a cichlid fish, *Geophagus brasiliensis*, there is continuous individual variation in habenular asymmetry that appears to be correlated with growth rate. Slow growing individuals tend to have leftward biased habenula, whereas faster growing individuals tend to have larger right habenula, and those with intermediate growth rates tended to have symmetrical habenulae. Furthermore, they found a positive correlation between strengths of the habenular and behavioural asymmetries. This represents the only study to date to look for a relationship between continuous natural variation in habenular lateralization and lateralization of behaviour. Natural variation in the asymmetry of the habenula within different species may provide an excellent opportunity to further investigate the correlation between habenular asymmetry and behavioural lateralization. The use of quantitative measurements of habenular asymmetry may help to clarify conflicting results about the relation between the direction of habenular and behavioural asymmetries [28].

The convict cichlid (*Amatitlania nigrofasciata*; formerly *Archocentrus nigrofaciatus*) is a monogamous and biparental Central American cichlid fish [36]. Recently, convicts have been employed as a model for the relation between individual variation in behavioural asymmetries and individual variation in personality-like characteristics. Reddon and Hurd [37] found that highly aggressive male convicts tend to be consistently asymmetrical in their behaviour while highly aggressive females tended to show weak or inconsistent lateral biases. Less-aggressive individuals showed the opposite pattern, with males being weakly biased in their behavioural lateralization and females showing a stronger, more consistent, bias. The relationship between behavioural asymmetry and boldness has also been investigated in convicts. Bolder individuals tend to be more consistently asymmetrical in their behaviour [38]. Male and female convicts also differ in their lateralization in response to emotionally conditioned stimuli. Males tend to show more consistent asymmetries in viewing negatively valent stimuli whereas females are more consistently lateralized in response to positively valent stimuli [39].

Here we investigate the relation between continuous individual variation of asymmetry in the habenula of wild-type convict cichlids and behaviour in the detour task, a commonly used test for behavioural lateralization in fish [37–44]. To our knowledge this will be the first attempt, to confirm the effects seen in Reddon et al. [35] in a different species. Convict cichlids are a widely employed model in the behavioural sciences and have recently been used as a model system in the study of comparative lateralization. We predict that individual variation in habenular asymmetry will correlate with individual performance in the detour task.

2. Materials and methods

2.1. Subjects

Subjects were adult convict cichlids purchased from local suppliers (22 females, 19 males). Prior to experimentation, subjects were housed together in a 440*L* (183 cm × 48 cm × 50 cm) aquarium with water temperature held constant at 25 ± 1 °C and a 12:12 h photoperiod. Fish were fed daily on a mixture of prepared cichlid foods. All fish were naïve to all testing procedures at the beginning of the study. All protocols were approved by the University of Alberta Biological Sciences Animal Policy and Welfare Committee (protocol #544706).

2.2. Behavioural testing

Fish were assayed for behavioural lateralization using the detour task ([40] described in detail in [37]). Briefly, the detour apparatus consisted of a large Plexiglas aquarium (195 cm × 30 cm × 29 cm) divided into two equal sections by a long narrow channel (75 cm). The fish was placed in one of the two compartments with the near end of the channel obstructed by a black Plexiglas door. A moveable barrier consisting of a Plexiglas sheet (15 cm across with alternating black and clear vertical bars approximately 0.75 cm wide creating a physical, but not visual, obstruction) was placed just beyond the distal end of the channel. At the start of a trial, the door was lifted and the fish was gently coaxed towards the opening of the channel using a dip-net. The fish would swim down the channel until it reached the bared barrier at which point it had to detour either left or right. A fish turning to the left fixates on the open area using its right eye while a right turning fish uses its left eye (as the opposite eye faces the opaque wall). Convicts, like most fish, have laterally placed eyes that project almost entirely to the contralateral hemisphere [45,46]. Measuring asymmetries in eye use is a well-established method for measuring asymmetries in hemisphere use in fish [42,46,47]. Each fish received ten trials, with successive trials run in opposite directions though the apparatus (as in [40,44]).

A behavioural laterality index (LI) for the each fish in the detour apparatus was calculated using the following formula:

$$\text{LI} = \frac{\text{right turns} - \text{left turns}}{\text{right turns} + \text{left turns}}$$

For example, a fish that made five left and five right turns would have an LI of zero, while one that made 10 left turns would have an LI of −1.

Analysing only the mean signed laterality index for a group of animals may obscure variation in the absolute strength of lateralization among the animals in the group because strongly lateralized animals with opposite detour preferences will cancel out when averaging [40]. Much previous work has suggested that the strength, rather than direction, of lateralization may be the more functionally important dimension [9,10,38,39,48,49]. Therefore, we calculated the absolute LI as a measure of an individual's strength of lateralization in the detour task.

2.3. Neuroanatomical measurements

Immediately following behavioural testing, fish were sacrificed by placing them in a 0.2% solution of 2-phenoxiethanol (p-1126, Sigma, St. Louis, MO). They were then decapitated and the head was immersed in 4% paraformaldehyde in phosphate buffer pH 7.4. After 2–3 weeks the brain was extracted from the skull, embedded in gelatin, and placed in 30% sucrose in 0.1 M PB for cryoprotection. Using a freezing-stage microtome, 30 μm sections were collected in 0.1 M phosphate buffered saline and mounted onto gelatinized slides. After air-drying, the slides were stained with thionine, dehydrated through a graded ethanol series, cleared in Hemo-D, and coverslipped with Permount.

Sections were viewed with a compound light microscope (Leica DMRE, Richmond Hill, ON). Images were acquired using a Retiga EXi *FAST* Cooled mono 12-bit camera (Qimaging, Burnaby, BC). Microphotographs of every section were taken throughout the rostrocaudal extent of the each of the habenula and a control area, the cortical pretectal nuclei (COPn). Measurements of the area of each side of each of the nuclei were taken directly from these photos using Image J (U.S. National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/). We calculated nucleus volumes by multiplying the area of the nuclei in each section by the thickness of the section (30 μm) and then adding them together. Neuroanatomical measures were made blind to the sex and behavioural score of the fish.

The cortical pretectal nucleus (COPn) was chosen as a control area because it is similar in size to the habenula and because it has clearly defined margins, characterized by large, densely packed cells. The COPn is a component of the pretectum and lays dorsomedial to the superficial pretectal zone. It has been shown to connect the optic tectum with the nucleus glomerularis in the caudal diencephalon, suggesting some role in visual behaviours [50].

Habenular measurements were taken from 18 males and 20 females. COPn measurements were taken from 17 males and 20 females. Some nuclei could not be measured on one, or both sides due to damage during preparation of the tissue, and thus not all of the 41 behaviour-tested individuals have complete brain asymmetry data.

One individual whose habenular asymmetry was 3.2 standard deviations from the mean (sd calculated with the outlier included) was discarded from the cerebral morphology analyses as an outlier, as was another individual whose COPn asymmetry was 3.4 sd from the mean (sd calculated with outlier included).

A laterality index (LI) was calculated for each nucleus in each individual using the following formula:

$$\mathrm{LI} = \frac{\text{volume right} - \text{volume left}}{\text{volume right} + \text{volume left}}$$

Similar indices have been used previously for measuring asymmetries in brain structures [51,52]. Absolute values were also computed in order to assess the overall strength of asymmetry.

3. Results

The convict cichlid habenula was very similar in structure to what has been previously reported for other species of cichlid fish (*Haplocromis burtoni* [50]; *G. brasiliensis* [35]). Briefly, it consisted of a cup-shaped protrusion into the third ventricle at the rostral-most portion of the diencephalon, with a thick layer of small, darkly staining cells against the ventricular wall and a more lateral neuropil. As in *H. burtoni* and *G. brasiliensis*, there was no apparent difference between the dorsal and ventral portions of the nucleus (Fig. 1).

We found no difference in the asymmetry of the habenula between that of males and females ($t = 0.69$, df = 26.8, $p = 0.49$; Figs. 2a and b, and 3a) and both had significantly larger left habenula (males: one-sample $t = -4.6$, df = 15, $p < 0.0005$; females: one-sample $t = -7.4$, df = 18, $p < 0.0001$).

Male and female convicts differed in their behaviour in the detour task ($t = -3.6$, df = 36.3, $p < 0.001$, Fig. 4a). Females exhibited a significant tendency to turn left and thus bring the open area into the right visual field while males preferentially turned right with their left eye outward (one-sample $t = 2.8$, df = 18, $p = 0.01$). Convict cichlids have laterally placed eyes that project almost entirely to the contralateral hemisphere, meaning that female convicts were primarily processing visual information with their left hemisphere while males were primarily using their right hemisphere when examining the space they were entering. We found no differences between males and females in the absolute strength of their lateral bias ($t = -0.32$, df = 30.7, $p = 0.75$) and both male and female convict cichlids are significantly biased at the individual level (males: one-sample $t = 5.1$, df = 18, $p < 0.0001$; females: one-sample $t = 7.5$, df = 21, $p < 0.0001$; Fig. 4b).

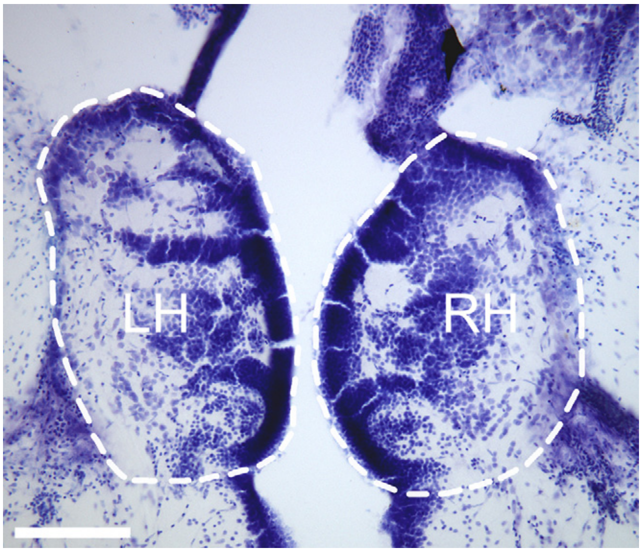

Fig. 1. Brightfield microphotograph of a coronal section through the habenula of a female convict cichlid (*Amatitlania nigrofasciata*). In this individual, the left habenula is 28.01% larger than the right. The laterality index is −0.123. Scale bar = 200 μm.

An ANCOVA with separate slopes analysis revealed a significant interaction between sex and habenular asymmetry on behavioural lateralization ($F(1,31) = 11.2$, $p < 0.01$, Fig. 5). In males the correlation is significant, and positive ($r = 0.62$, df = 13, $p = 0.01$). In females, behavioural lateralization shows no significant correlation with habenular asymmetry ($r = -0.27$, df = 15, $p = 0.30$). As mentioned above, we found a significant bias towards larger left habenula in both sexes, and only a few individuals presented right biased habenula. As a result, males with symmetrical or slightly right biased habenula tend to turn right, with their left eye out and use their right hemisphere for processing the visual information. Even though the correlation was not significant, females appeared to show the opposite tendency, with individuals whose habenulae were right biased or relatively symmetrical habenulae tending to turn left and hence use their left hemisphere. An ANCOVA with separate slopes analysis showed no effect of habenular asymmetry or sex in absolute LI, the strength of behavioural lateralization ($F(3,28) = 1.742$, $p = 0.1812$).

We measured the COPn as a control nucleus and found that the average absolute laterality index of the habenula (0.075 ± 0.008) was significantly larger than in the COPN (0.042 ± 0.005; $t = -5.6$, df = 33, p-value $\ll 0.0001$) and found no correlation between the laterality index of the habenula and the COPn ($r = -0.27$, df = 32, $p = 0.12$). There was no relation between COPn LI and behavioural lateralization ($r = 0.04$, df = 37, $p = 0.84$).

4. Discussion

In this study we found that in both male and female convict cichlids there is a significant population bias towards a relatively larger left habenula. Within this pattern, we found a large variation in the magnitude of the asymmetry, with some individuals showing relatively symmetrical habenula and even some rightward biased individuals (see Fig. 2). We found that the habenula is significantly more asymmetrical than our control region, the COPn (Fig. 2). Furthermore, asymmetries in the Hb and the COPn were uncorrelated, indicating that the differences between left and right side are exclusive to the habenula and not part of a more general brain asymmetry.

Previously, Reddon et al. [35] had found in another cichlid fish (*G. brasiliensis*) that, individuals showed an asymmetry of the habenula of similar magnitude, but found no population bias in the direction of this asymmetry. Fernald and Shelton [50] found that in a qualitative assessment of a small sample of the African cichlid *H. burtoni*, the right habenula appeared slightly larger. Collectively, these results suggest that the cichlid family presents a large variation of habenular asymmetries.

The developmental mechanism leading to habenular asymmetry has been extensively studied in zebrafish (reviewed in [53]) but little is known about what factors may be responsible for interspecific variation in habenular asymmetries. Reddon et al. [35] showed that in *G. brasiliensis*, the habenular asymmetry is correlated with growth rate. Slow growing individuals present leftward biased habenulae while fast growing individuals exhibited

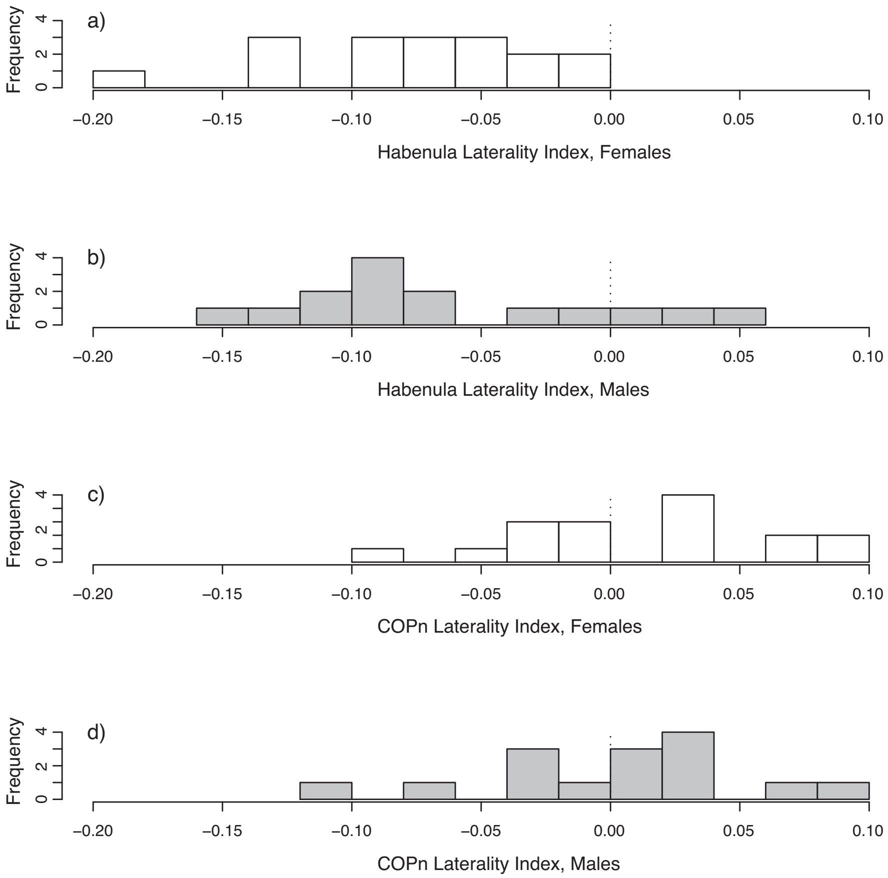

Fig. 2. Distributions of laterality indices for the habenula and COPn by sex. (a) = Hb LI for males; (b) = Hb Li for females; (c) = COPn LI for males; (d) = COPn LI for females.

rightward biases. We are not able to speak to the potential effects of growth rate on habenula asymmetry in convicts as the ages, and hence growth rates of the fish in this study were not known.

In zebrafish, the position of the parapineal organ is a major determinant of habenular asymmetry [54]. In teleosts, this unpaired structure projects exclusively to the left habenula [31,55]. In zebrafish, it has been shown that ablation of the parapineal organ during development results in symmetrical habenula [56]. In both zebrafish and medaka (*Oryzias latipes*), it has been shown that the areas in the left habenula that are innervated by parapineal axons become increasingly complex and differentiate from their right side counterpart [57,58]. Furthermore, it has been suggested that assignment of parapineal position to the left might be implicated in the asymmetric regulation of neurogenesis in the habenula [59]. It is likely that both intra- and interspecific differences in habenular asymmetry are related to the influence of the parapineal organ. In fish, the parapineal organ is a directly photosensitive structure (reviewed in [60]). In birds and mammals, it is well established that light-dependent activity is essential for the normal development of the main visual pathways ([61] and references within). Is possible that a similar mechanism operates during the development of the parapineal-habenula pathway and that differences in light exposure during development would influence the development of habenular asymmetries. Consistent with this line of reasoning, Budaev and Andrew [62] found that altering exposure to light during development in zebrafish affected the expression of behavioural lateralization later in life.

We also found that habenular asymmetry is correlated to behavioural lateralization. Males with more symmetrical habenula showed a significant bias for right turns, while females showed a significantly different relationship, with more symmetrical habenula being associated with an apparent bias towards left turns. Males with more symmetrical habenula therefore generally processed information about the open area with the right hemisphere as it lies within the left visual field, and females with the left hemisphere. We found no differences between males and females on the strength of habenular asymmetry or behavioural lateralization. Therefore, a male and a female with equally asymmetric habenula presented the same magnitude of behavioural lateralization but in opposite directions. Reddon et al. [35] found that in *G. brasiliensis* males, the strength, but not the direction, of habenular asymmetry is correlated with the strength of turn bias in this same task.

The complexity of the interaction between behavioural asymmetry and habenular asymmetry may be explained in part by the topography of the habenular afferents. The main target of the habenula is the interpeduncular nucleus (IPN), a group of cells in the ventral midbrain [15]. In the zebra fish and in the southern flounder (*Paralichthys lethostima*), tracing studies have shown that each side of the habenula projects bilaterally to IPN, but that the left habenula projects preferentially to the dorsal part of IPN, while the right habenula projects preferentially to the ventral part [63–65]. In the zebra fish, the IPN is characterized by two kinds of neurons, the first type have neurites restricted to either the dorsal or ventral neuropil and therefore contact inputs only from the right or left habenula, whereas the second type extend their neurites to both dorsal and ventral neuropils thus integrating left and right inputs [66]. Recent works by Agetsuma et al. [67] have shown that the dorsal and ventral IPN project to different regions of the raphe nucleus. This implies that lateralized inputs from the habenula can be kept separated further downstream and/or integrated at the same time.

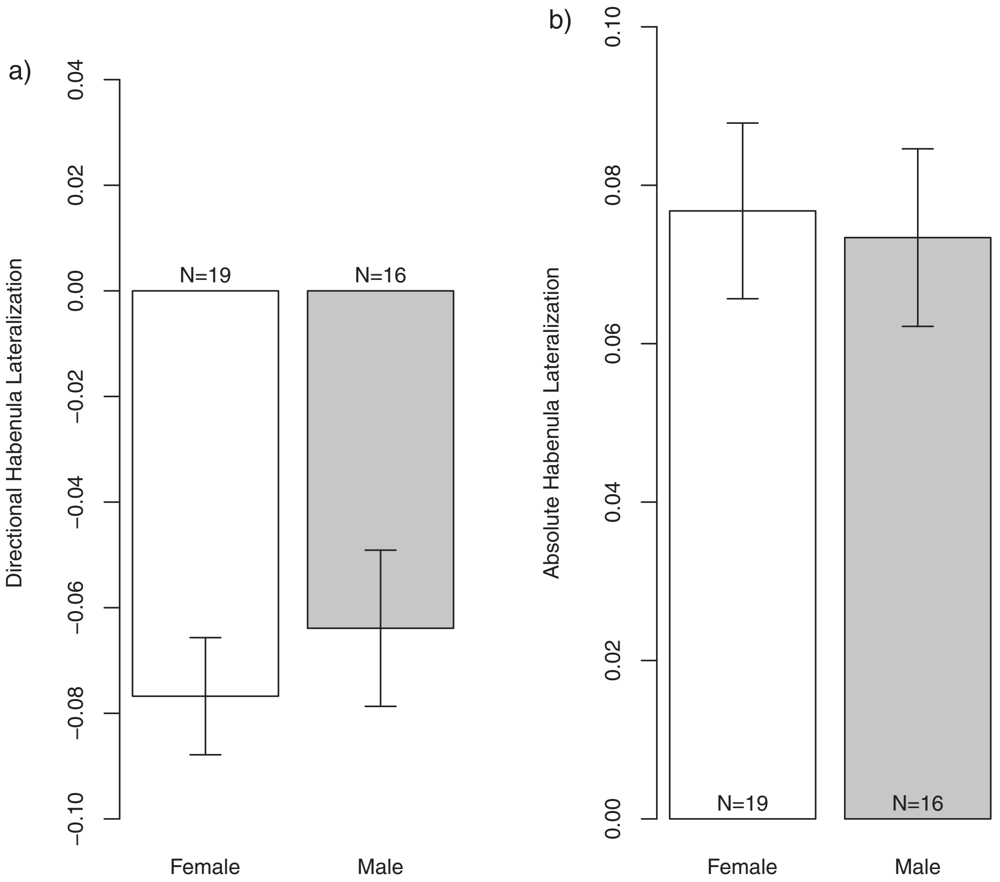

Fig. 3. (a) Mean ± SE habenular laterality index for *A. nigrofasciata*. Positive scores indicate a larger right habenula; negative scores indicate a larger left habenula. (b) Mean ± SE absolute habenular laterality index.

However, it is unknown how the habenulo-IPN-raphe pathway interacts with other conduction systems to produce lateralization of behaviour [34].

The sex-specific relation between the direction of asymmetry in the habenula and the direction of behavioural lateralization found in convicts cichlids (this study) and the indirect relation observed in *G. brasiliensis* [35] contrasts with what has been found in studies using zebrafish. Barth et al. [32] showed that in *fsi* zebrafish, in which a high percentage of individuals present *situs inversus* (resulting in reversal of asymmetries in viscera and brain, including the habenula), individuals present also reversal of some, but not all, lateralized behaviours. Facchin et al. [34] found that artificial selection for behavioural lateralization affected the frequency of individuals with reversed asymmetry in the habenula. Artificial selection for increased right eye (left hemisphere) use in a mirror image inspection task resulted in increased proportions of individuals with directionally reversed neural symmetry in subsequent generations. More recently Dadda et al. [33] has shown that individuals with reversed asymmetry in the epithalamus show marked difference from normal individuals in several tasks measuring behavioural lateralization and personality-like traits, such as boldness. One potential issue with these studies in zebrafish is that in all cases the reversal of the asymmetry of the habenula is part of a general reversal of the left-right body axis development. This means that the asymmetry of other regions or circuits of the brain that may be responsible behavioural lateralization would be also reversed, obscuring the role of habenular asymmetry on the reversal of behavioural lateralization. Furthermore, the aforementioned studies looked at the relation between behaviour and the categorical direction of habenular asymmetry. The current study is one of only two studies that have looked for the relation between lateralized behaviour and natural continuous variation in habenular asymmetry.

The sex differences in the interaction between habenular asymmetry and behavioural lateralization are consistent with previous findings in convict cichlids showing differences in lateralized behaviours between males and females. Reddon and Hurd [37] found an interaction between sex, aggressiveness and behavioural lateralization. These authors showed that while relatively non-aggressive females tented to turn right in a detour task and female aggressors turned left, the opposite was true for males. Furthermore, the strength of lateralization was also influenced by sex and aggressiveness. Female non-aggressors were more strongly lateralized than male non-aggressors but male aggressors were more strongly lateralized than female aggressors.

In a subsequent study, the same authors [39] showed that female convict cichlids have a stronger preference for viewing positively conditioned stimuli with one particular eye, whereas males have a stronger eye preference when viewing negatively conditioned stimuli. Recently, Arnott and Elwood [68] found that during intrasexual agonistic encounters, female convicts tended to use more frontal display whereas males used more lateral display, suggesting differences in eye use with regard to aggressive behaviours.

As mentioned above, we found no differences in the asymmetry of the habenula between males and females. Therefore, sex differences in behavioural lateralization observed in the convict cichlid cannot be explained by a direct, universal correspondence with habenular laterality. It is possible that another region or regions of the brain is responsible for the lateralization of behaviour and the habenulo-IPN pathway interacts with this region(s). Another possible explanation for the sex differences in the relation between

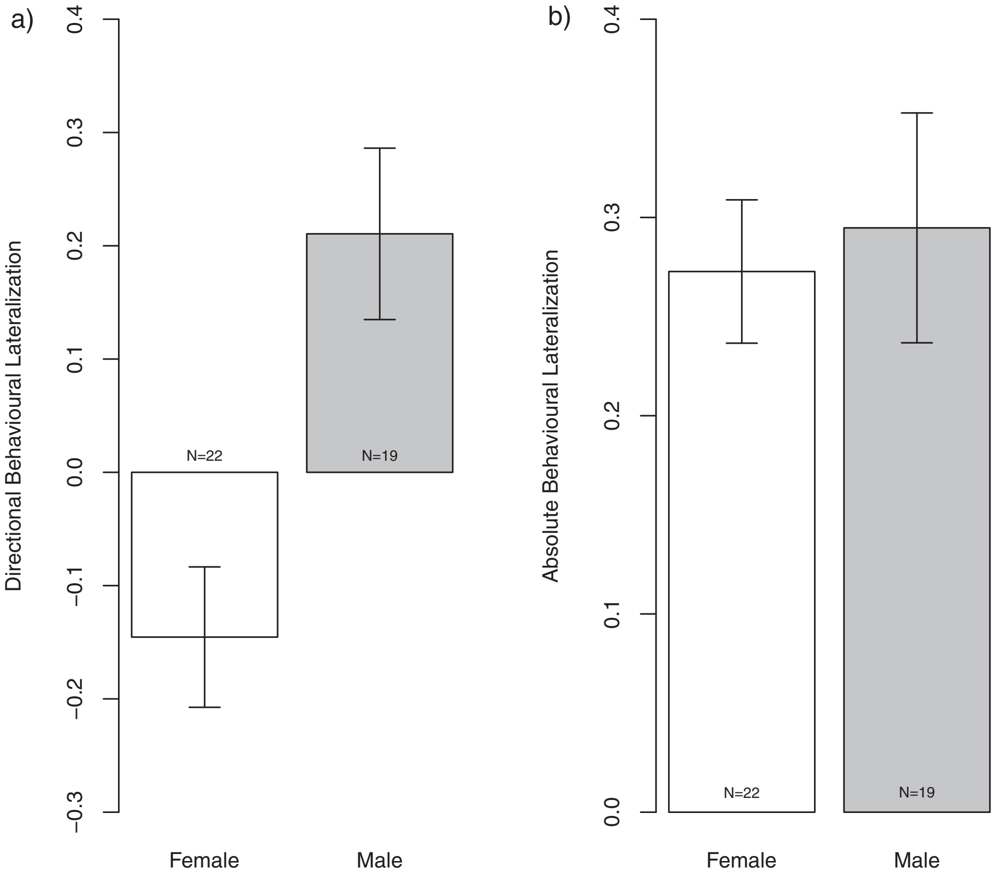

Fig. 4. (a) Mean ± SE behavioural laterality index for *A. nigrofasciata*. Positive scores indicate a right detour bias; negative scores indicate a left detour bias. (b) Mean ± SE absolute behavioural laterality index.

habenular and behavioural asymmetries that we observed is that male and female convicts exhibit different motivations in the detour task. The habenula has been shown to play a central role in the control of motivation in mammals by inhibiting dopaminergic circuits downstream, which in turn, inhibits motor behaviour (reviewed in [17]). It is not known whether the habenula also shares this function in fish, but there is some evidence to suggest that it may [22]. Differences in motivation are known to affect the pattern of lateralization observed in other species of fish, for example sexually deprived female poeciliid fish show a different pattern of lateralized eye use when viewing male conspecifics than those that have recently been mated, which has been attributed to differences in sexual motivation [41]. If male and female convicts interpret our detour task differently and have different motivations when behaving in it, this may explain the sex specific concordance between brain asymmetry and detour direction that we observed. The fact that male and female convicts showed on average different detour directions (males turned right and females turned left) might lend some support for this interpretation. It is possible that the habenula does not play any direct role in behavioural lateralization and that the correlations between asymmetry of the habenula and asymmetry of behaviour observed in the current study and in previous studies owes entirely to the role the habenula plays in the motivational system. Determining whether the habenula plays a direct role in functional lateralization or an indirect one through its effects on motivation is an important direction for future research.

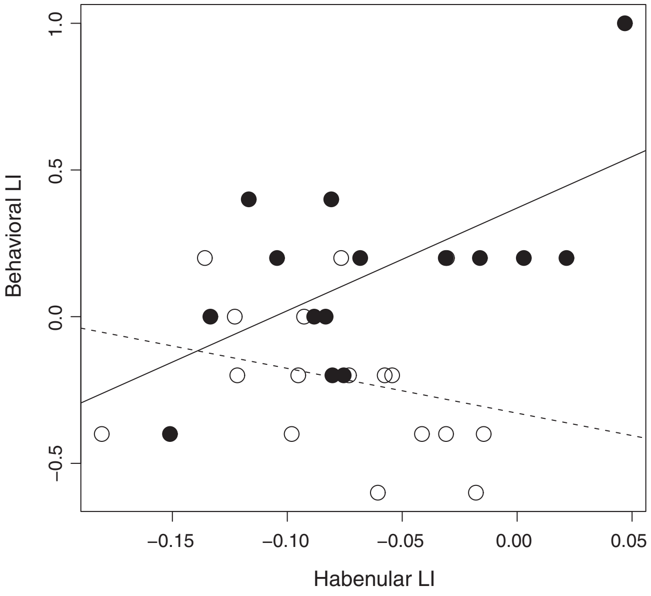

Fig. 5. The linear relationship between habenular laterality index and behavioural laterality index in *A. nigrofasciata*. Males: closed circles and solid line; females: open circles and dashed line (males: $p = 0.01$; females: $p = 0.30$, sex by habenular LI interaction: $p < 0.01$).

This study illustrates the importance of a comparative approach to the study of the habenular asymmetry and its relation to behaviour. Cichlids have gone through large adaptive radiations, both in Africa and in the Americas, totaling about 2200 species, filling a diverse array of ecological niches and exhibiting an expansive variety of social behaviours [69,70]. This could make cichlids an excellent model group to study the evolution and development of habenular asymmetry and its relation to behaviour.

Acknowledgements

We thank Isaac Lank for constructing the detour apparatus and Kathryn Vaillancourt for assistance in data collection and animal care. This research was funded by a National Science and Engineering Research Council (NSERC) discovery grants to PLH and DRW. ARR is supported by an NSERC Canada Graduate Scholarship (Doctoral).

References

[1] Rogers LJ, Andrew RJ. Comparative vertebrate lateralization. Cambridge: Cambridge University Press; 2002.
[2] Vallortigara G, Rogers LJ. Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. Behav Brain Sci 2005;28: 575–89.
[3] MacNeilage PF, Rogers LJ, Vallortigara G. Origins of the left and right brain. Sci Am 2009;(July):60–7.
[4] Vallortigara G. Comparative neuropsychology of the dual brain: a stroll through left and right animals' perceptual worlds. Brain Lang 2000;73:189–219.
[5] Vallortigara G. Cerebral lateralization: a common theme in the organization of the vertebrate brain. Cortex 2006;42:5–7.
[6] Vallortigara G. The evolutionary psychology of left and right: costs and benefits of lateralization. Dev Psychobiol 2006;48:418–27.
[7] Magat M, Brown C. Laterality enhances cognition in Australian parrots. Proc Biol Sci 2009;276:4155–62.
[8] Rogers LJ, Zucca P, Vallortigara G. Advantages of having a lateralized brain. Proc R Soc Lond B (Suppl) 2004;271:S420–2.
[9] Dadda M, Bisazza A. Does brain asymmetry allow efficient performance of simultaneous tasks? Anim Behav 2006;72:523–9.
[10] Dadda M, Bisazza A. Lateralized female topminnows can forage and attend to a harassing male simultaneously. Behav Ecol 2006;7:358–63.
[11] Vallortigara G, Chiandetti C, Sovrano VA. Brain asymmetry (animal). WIREs Cogni Sci 2011;2:146–57.
[12] Güntürkün O. Avian visual lateralization: a review. Neuroreport 1997;8, iii–xi.
[13] Rogers LJ, Deng C. Light experience and lateralization of the two visual pathways in the chick. Behav Brain Res 1999;98:277–87.
[14] Skiba M, Diekamp B, Güntürkün O. Embryonic light stimulation induces different asymmetries in visuoperceptual and visuomotor pathways of pigeons. Behav Brain Res 2002;134:149–56.
[15] Sutherland RJ. The dorsal diencephalic conduction system: a review of the anatomy nd functions of the habenular complex. Neurosci Biobehav Rev 1982;6:1–13.
[16] Bianco IH, Wilson SW. The habenular nuclei: a conserved asymmetric relay station in the vertebrate brain. Philos Trans R Soc Lond B Biol Sci 2009;364:1005–20.
[17] Hikosaka O. The habenula: from stress evasion to value-based decision making. Nat Rev Neurosci 2010;11:503–13.
[18] Sandyk R. Relevance of the habenular complex to neuropsychiatry: a review and hypothesis. Int J Neurosci 1991;61:189–219.
[19] Klemm WR. Habenular and interpeduncularis nuclei: shared components in multiple-function networks. Med Sci Monit 2004;10:261–73.
[20] Hikosaka O, Sesack S, Lecourtier L, Shepard P. Habenula: crossroad between the basal ganglia and the limbic system. J Neurosci 2008;28:11825–9.
[21] Matsumoto M, Hikosaka O. Lateral habenula as a source of negative reward signals in dopamine neurons. Nature 2007;447:1111–5.
[22] Amo R, Aizawa H, Takahoko M, Kobayashi M, Takahashi R, Aoki T, et al. Identification of the zebrafish ventral habenula as a homolog of the mammalian lateral habenula. J Neurosci 2010;30:1566–74.
[23] Concha ML, Wilson SW. Asymmetry in the epithalamus of vertebrates. J Anat 2001;199:63–84.
[24] Cole WC, Youson JH. Morphology of the pineal complex of the anadromous sea lamprey, *Petromyzon marinus* L. Am J Anat 1982;165:131–63.
[25] Nieuwenhuys R. Chondrostean fishes. In: Nieuwenhuys R, ten Donkelaar HJ, Nicholson C, editors. The central nervous system of vertebrates, vol. 1. Berlin: Springer; 1998. p. 701–58.
[26] Smeets WJ. The secondary olfactory connections in two chondrichthians, the shark *Scyliorhinus canicula* and the ray *Raja clavata*. J Comp Neurol 1983;218:334–44.
[27] Concha ML. The dorsal diencephalic conduction system of zebrafish as a model of vertebrate brain lateralisation. Neuroreport 2004;15:1843–6.
[28] Facchin L, Argenton F, Bisazza A. Lines of *Danio rerio* selected for opposite behavioural lateralization show differences in anatomical left-right asymmetries. Behav Brain Res 2009;197:157–65.
[29] Andrew RJ, Dharmaretnam M, Gyori B, Miklósi A, Watkins JA, Sovrano VA. Precise endogenous control of involvement of right and left visual structures in assessment by zebrafish. Behav Brain Res 2009;196:99–105.
[30] Sovrano VA, Andrew RJ. Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae. Behav Brain Res 2006;167: 226–31.
[31] Concha ML, Burdine RD, Russell C, Schier AF, Wilson SW. A nodal signaling pathway regulates the laterality of neuroanatomical asymmetries in the zebrafish forebrain. Neuron 2000;28:399–409.
[32] Barth KA, Miklosi A, Watkins J, Bianco IH, Wilson SW, Andrew RJ. fsi zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses. Curr Biol 2005;15:844–50.
[33] Dadda M, Domenichini A, Piffer L, Argenton F, Bisazza A. Early differences in epithalamic left-right asymmetry influence lateralization and personality of adult zebrafish. Behav Brain Res 2010;206:208–15.
[34] Facchin L, Burgess HA, Siddiqi M, Granato M, Halpern ME. Determining the function of zebrafish epithalamic asymmetry. Philos Trans R Soc Lond B Biol Sci 2009;64:1021–32.
[35] Reddon AR, Gutiérrez-Ibáñez C, Wylie DR, Hurd PL. The relationship between growth, brain asymmetry and behavioural lateralization in a cichlid fish. Behav Brain Res 2009;201:223–8.
[36] Wisenden BD. Reproductive behavior of free-ranging convict cichlids. *Cichlasoma nigrofasciatum*. Environ Biol Fish 1995;43:121–34.
[37] Reddon AR, Hurd PL. Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. Biol Lett 2008;4:338–40.
[38] Reddon AR, Hurd PL. Individual differences in cerebral lateralization are associated with shy-bold variation in the convict cichlid. Anim Behav 2009;77:189–93.
[39] Reddon AR, Hurd PL. Sex differences in the cerebral lateralization of a cichlid fish when detouring to view emotionally conditioned stimuli. Behav Processes 2009;82:25–9.
[40] Bisazza A, Pignatti R, Vallortigara G. Laterality in detour behaviour: interspecific variation in poeciliid fish. Anim Behav 1997;54:1273–81.
[41] Bisazza A, Facchin L, Pignatti R, Vallortigara G. Lateralization of detour behaviour in poeciliid fish: the effect of species, gender and sexual motivation. Behav Brain Res 1998;91:157–64.
[42] Facchin L, Bisazza A, Vallortigara G. What causes lateralization in detour behaviour in fish? Evidence for asymmetries in eye use. Behav Brain Res 1999;103:229–34.
[43] Kaarthigeyan J, Dharmaretnam M. Relative levels of motivation and asymmetries of viewing and detour task in guppies (*Poecilia reticulata*). Behav Brain Res 2005;159:37–41.
[44] Brown C, Western J, Braithwaite VA. The influence of early experience on, and inheritance of, cerebral lateralization. Anim Behav 2007;74:231–8.
[45] Bisazza A, Rogers LJ, Vallortigara G. The origins of cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. Neurosci Biobehav Rev 1998;3:411–26.
[46] Vallortigara G, Bisazza A. How ancient is brain lateralization? In: Rogers LJ, Andrew RJ, editors. Comparative vertebrate lateralization. Cambridge: Cambridge University Press; 2002. p. 9–69.
[47] Sovrano VA, Rainoldi C, Bisazza A, Vallortigara G. Roots of brain specializations: preferential left-eye use during mirror image inspection in six species of teleost fish. Behav Brain Res 1999;106:175–80.
[48] Bisazza A, Dadda M. Enhanced schooling performance in lateralized fishes. Proc R Soc B 2005;272:1677–81.
[49] Sovrano VA, Dadda M, Bisazza A. Lateralized fish perform better than nonlateralized fish in spatial reorientation tasks. Behav Brain Res 2005;163: 122–7.
[50] Fernald RD, Shelton LC. The organization of the diencephalon and the pretectum in the cichlid fish *Haplochromis burtoni*. J Comp Neurol 1985;238: 202–17.
[51] Van Eden CG, Uylings HBM, Van Pelt J. Sex differences and left-right asymmetries in the prefrontal cortex during postnatal development in the rat. Dev Brain Res 1984;12:146–53.
[52] Gurusinghe CJ, Zappia JV, Ehrlich D. The influence of testosterone on the sex-dependent structural asymmetry of the medial habenular nucleus of the chicken. J Comp Neurol 1986;253:153–62.
[53] Concha ML, Signore IA, Colombo A. Mechanisms of directional asymmetry in the zebrafish epithalamus. Semin Cell Dev Biol 2009;20:498–509.
[54] Guglielmotti V, Cristino L. The interplay between the pineal complex and the habenular nuclei in lower vertebrates in the context of the evolution of cerebral asymmetry. Brain Res Bull 2006;69:475–88.
[55] Yanez J, Meissl H, Anadon R. Central projections of the parapineal organ of the adult rainbow trout (*Oncorhynchus mykiss*). Cell Tissue Res 1996;285:69–74.
[56] Gamse JT, Thisse C, Thisse B, Halpern ME. The parapineal mediates left-right asymmetry in the zebrafish diencephalon. Development 2003;130:1059–68.
[57] Concha ML, Russell C, Regan JC, Tawk M, Sidi S, Gilmour DT, et al. Local tissue interactions across the dorsal midline of the forebrain establish CNS laterality. Neuron 2003;39:423–38.
[58] Signore IA, Guerrero N, Loosli F, Colombo A, Villalon A, Wittbrodt J, et al. Zebrafish and medaka: model organisms for a comparative developmental approach of brain asymmetry. Philos Trans R Soc Lond B Biol Sci 2009;364:991–1004.
[59] Aizawa H, Goto M, Sato T, Okamoto H. Temporally regulated asymmetric neurogenesis causes left-right difference in the zebrafish habenular structures. Dev Cell 2007;12:87–98.
[60] Ekström P, Meissl H. Evolution of photosensory pineal organs in new light: the fate of neuroendocrine photoreceptors. Philos Trans R Soc Lond B Biol Sci 2003;358:1679–700.
[61] Prichard JR, Armacanqui HS, Benca RM, Behan M. Light-dependent retinal innervation of the rat superior colliculus. Anat Rec (Hoboken) 2007;290: 341–8.
[62] Budaev S, Andrew RJ. Patterns of early embryonic light exposure determine behavioural asymmetries in zebrafish: a habenular hypothesis. Behav Brain Res 2009;200:91–4.

[63] Aizawa H, Bianco IH, Hamaoka T, Miyashita T, Uemura O, Concha ML, et al. Laterotopic representation of left-right information onto the dorso-ventral axis of a zebrafish midbrain target nucleus. Curr Biol 2005;15:238–43.

[64] Gamse JT, Kuan YS, Macurak M, Brösamle C, Thisse B, Thisse C, et al. Directional asymmetry of the zebrafish epithalamus guides dorsoventral innervation of the midbrain target. Development 2005;132:4869–81.

[65] Kuan YS, Gamse JT, Schreiber AM, Halpern ME. Selective asymmetry in a conserved forebrain to midbrain projection. J Exp Zool B Mol Dev Evol 2007;308:669–78.

[66] Bianco IH, Carl M, Russell C, Clarke JD, Wilson SW. Brain asymmetry is encoded at the level of axon terminal morphology. Neural Dev 2008;1:3–9.

[67] Agetsuma M, Aizawa H, Aoki T, Nakayama R, Takahoko M, Goto M, et al. The habenula is crucial for experience-dependent modification of fear responses in zebrafish. Nat Neurosci 2010;13:1354–6.

[68] Arnott G, Elwood RW. Gender differences in aggressive behaviour in convict cichlids. Anim Behav 2009;78:1221–7.

[69] Turner GF. Explosive speciation of African Cichlid fishes. In: Magurran AE, May RM, editors. Evolution of biological diversity. Oxford: University Press; 1999. p. 113–29.

[70] Turner GF. Adaptive radiation of cichlid fish. Curr Biol 2007;17:R827–31.