Left and right in the amphibian world: which way to develop and where to turn?

Yegor B. Malashichev[1]* and Richard J. Wassersug[2]

Summary

The last decade has seen a dramatic increase in studies on the development, function and evolution of asymmetries in vertebrates, including amphibians. Here we discuss current knowledge of behavioral and anatomical asymmetries in amphibians. Behavioral laterality in the response of both adult and larval anurans to presumed predators and competitors is strong and may be related, respectively, to laterality in the telencephalon of adults and the Mauthner neurons of tadpoles. These behavior lateralities, however, do not seem to correlate with visceral asymmetries in the same animals. We briefly compare what is known about the evolution and development of asymmetry in the structure and function of amphibians with what is known about asymmetries in other chordate and non-chordate groups. Available data suggest that the majority of asymmetries in amphibians fall into two independent groups: (1) related to situs viscerum and (2) of a neurobehavioral nature. We find little evidence linking these two groups, which implies different developmental regulatory pathways and independent evolutionary histories for visceral and telencephalic lateralizations. Studies of animals other than standard model species are essential to test hypotheses about the evolution of laterality in amphibians and other chordates. *BioEssays* **26:512–522, 2004.**
© 2004 Wiley Periodicals, Inc.

Introduction

The evolution and development of left–right asymmetry in otherwise bilateral organisms is an important area of developmental and neurobiology. Behavioral asymmetries, which were conventionally thought of as unique to humans and other higher vertebrates, are in fact common among vertebrates.[1–3] Although the study of asymmetry in vertebrates has a long history dating back to the early 20th century,[4] much recent evidence has accumulated on the development and function of asymmetries in lower vertebrates, especially in amphibians (see Ref. 5, 6 and references therein). These new data on amphibians can be compared to the conceptual framework for left–right patterning of other animals to provide new perspectives, which is the goal of this paper.

Here we summarize the current knowledge of asymmetries in amphibians, with special reference to their development and evolution. We consider "asymmetry" to be the state of an organism when its two sides are unlike, or disproportionate, in structure. We use "lateralization" for functional asymmetries. We will discuss the relationships of structural and functional asymmetries at both individual and population levels. In the latter case, population asymmetry or lateralization would be a prevalence of one of the two asymmetric states (left or right) in a group of organisms.

Two major conclusions that emerge from this review are that behavioral asymmetries can be more variable in populations and species than the principal body left–right asymmetries, and not obviously coupled to them in development and evolution.

Brain lateralization in amphibians is similar to that of other vertebrates

There is a common pattern for cerebral hemispheric specialization[2,7] that has been documented in all vertebrate classes. The right hemisphere is associated with rapid responses, expression of intense emotions, parallel processing and attention to spatial information. Thus, the right hemisphere dominates behaviors including predator escape, expression of fear, aggression and sexual behavior. When behavior (e.g., aggressiveness to a conspecific) is lateralized, it is usually exhibited on the left side of the organism.

The left hemisphere, in contrast, controls more considered (slow) responses and performs sequential analysis. Feeding and prey capture behaviors are typically left hemisphere specializations and when lateralized, they are usually directed toward the right.

Although cerebral lateralization is not always evident at the population level, in behavioral tests it is usually present among

[1]Department of Vertebrate Zoology, St. Petersburg State University, Russia.

[2]Department of Anatomy and Neurobiology, Dalhousie University, Halifax, Canada.

*Correspondence to: Yegor B. Malashichev, Department of Vertebrate Zoology, St. Petersburg State University, Universitetskaya nab, 7/9, St. Petersburg, 199034, Russia. E-mail: Yegor@EM1915.spb.edu

individuals. Hence the absence of one-sided population preferences in selected behavioral tests cannot be construed as a lack of lateralization in general.[8]

The strongest evidence of hemispheric specialization in amphibians comes from observations of the interactions of adult toads with each other while feeding.[9,10] Toads of the genus *Bufo* (*Bufo bufo, B. viridis*, and *B. marinus*) strike earlier at prey that appears in their right rather than left visual hemifield, suggesting control by the left cerebral hemisphere. In contrast, aggressive strikes toward conspecifics are preferentially made toward animals on the toads' left side. A more recent study,[11] in which a model predator was presented frontally or laterally to adult toads of those same three species, revealed stronger escape and defensive reactions when the model first appeared in each animal's left visual hemifield; i.e., the response was driven by the right hemisphere.
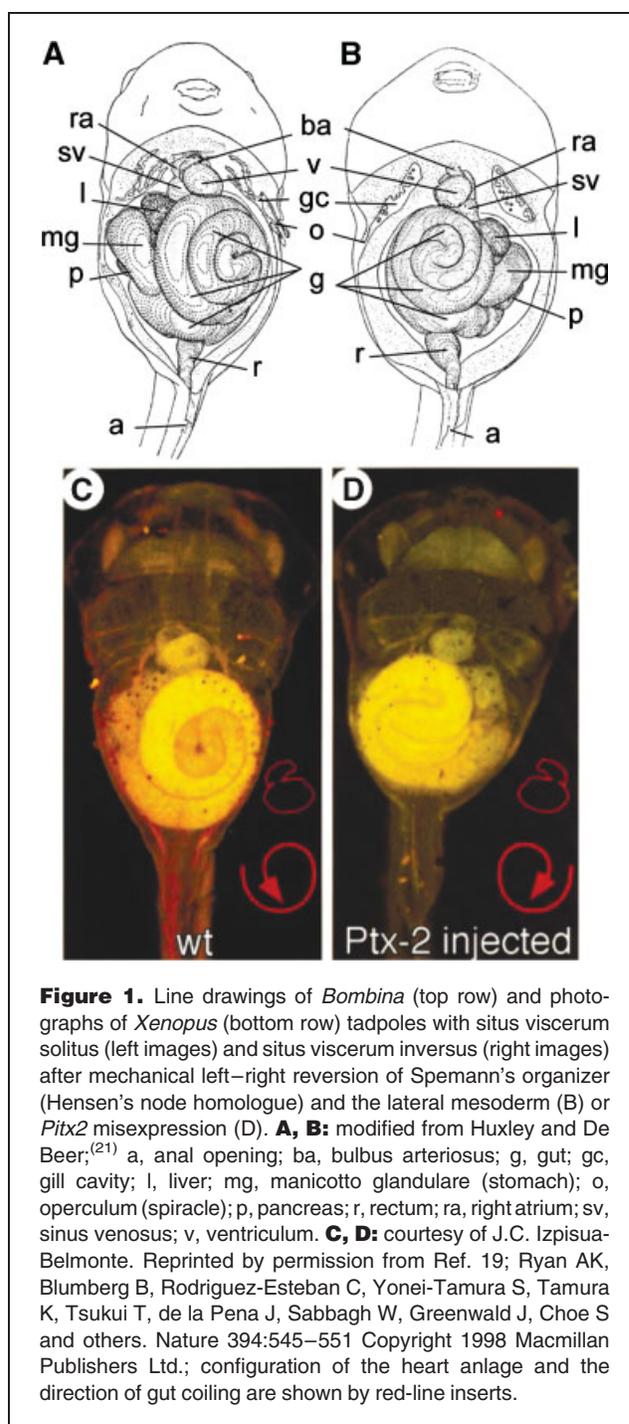
There is some recent evidence that anuran larvae similarly prefer to view each other with their left eye.[12] This is reminiscent of the left-eye preference found in schooling fish[13] and has been interpreted in terms of hemispheric specialization related to both adaptation to predator escape and sociality.[3,7,14]

Population lateralization of sexual behavior occurs in at least one caudate amphibian, *Triturus vulgaris*.[15] Here males more readily turn left than right during spermatophore transfer to the female. This finding suggests a visually guided, lateralized behavior, again reflecting specializations of the hemispheres. However, this lateralized behavior was not found in two other *Triturus* species, *T. alpestris* and *T. cristatus*.[15,16]

A number of hypotheses on the evolutionary origin of hemispheric specialization are suggested and discussed elsewhere.[3,17,18] Here we focus on the links between these asymmetries of the neural system and subsequent behavior. Where possible, we also explore how these asymmetries may have developed.

Gene control over development of morphological asymmetry

The major viscera in vertebrates are asymmetrical. The vast majority of individuals show a rightward looping of the heart anlage, asymmetry in the lung and liver lobes, and left-sided placement of the stomach and spleen. In anuran larvae, the gut is normally coiled counterclockwise,[19] while the spiracle—the out-current opening for water that has passed over the gills in tadpoles—when single, is usually situated on the left side of the body.[20] The kidneys, gonads and fat bodies are also asymmetric in tadpoles, with the right kidneys larger and the fat bodies more extensive on the left than the right side. Such an asymmetric placement of viscera (situs viscerum solitus, Fig. 1A,C) can occasionally be reversed[19,21] (situs viscerum inversus, Fig. 1B,D).

Figure 1. Line drawings of *Bombina* (top row) and photographs of *Xenopus* (bottom row) tadpoles with situs viscerum solitus (left images) and situs viscerum inversus (right images) after mechanical left–right reversion of Spemann's organizer (Hensen's node homologue) and the lateral mesoderm (B) or *Pitx2* misexpression (D). **A, B:** modified from Huxley and De Beer;[21] a, anal opening; ba, bulbus arteriosus; g, gut; gc, gill cavity; l, liver; mg, manicotto glandulare (stomach); o, operculum (spiracle); p, pancreas; r, rectum; ra, right atrium; sv, sinus venosus; v, ventriculum. **C, D:** courtesy of J.C. Izpisua-Belmonte. Reprinted by permission from Ref. 19; Ryan AK, Blumberg B, Rodriguez-Esteban C, Yonei-Tamura S, Tamura K, Tsukui T, de la Pena J, Sabbagh W, Greenwald J, Choe S and others. Nature 394:545–551 Copyright 1998 Macmillan Publishers Ltd.; configuration of the heart anlage and the direction of gut coiling are shown by red-line inserts.

The underlying mechanism that determines body asymmetry has been recently proposed as common for all vertebrates.[22,23] Fluid flow, caused by the circular beating of cilia in the Hensen's node, triggers a left unilateral expression of *nodal* (with bone morphogenetic protein acting on the right side) in the mouse.[22] Although nodal cilia occur in all major groups of vertebrates,[23] their function in creating the

fluid flow and relevance to establishing visceral asymmetries has been questioned.[24,25] Other regulatory pathways for generating left−right asymmetry are possible such as those involving gap-junction communication plus early asymmetry in ion flux and cell membrane potentials in the pre-node chick and four-cell *Xenopus* embryos.[24,26,27]

Nevertheless, the Nodal-regulated gene cascade (particularly *Pitx* genes), no matter how it is established, is conserved in all major groups of vertebrates. Its expression and function in primitive chordates, like amphioxus, is similar to that of higher vertebrates. This suggests that these genes form the stable core for an ancient mechanism regulating visceral asymmetry.[28]

This molecular pathway is also involved in the control of other aspects of embryo asymmetry. In amniotes, a subtle twisting of the body, which starts in the head region with a more dorsal exposure of the right eye and ear,[29] occurs in addition to visceral asymmetries. Contrary to amniotes, which show strong population bias in embryo rotation, amphibian embryos may not be lateralized in this respect.

Molecular mechanisms underlying the development of visceral asymmetries may relate to brain structure. Asymmetry in the habenular nuclei in the diencephalon occurs in lampreys, birds and fish. In fish, the Nodal-regulated pathway determines habenular and parapineal asymmetry.[30,31] The habenulae in anuran and caudate amphibians are also lateralized; i.e., greater on the left side, and this asymmetry has a population bias that coincidentally correlates with situs viscerum.[33,34] Unfortunately, the functions of the habenulae and the significance of their asymmetry in amphibians are still uncertain,[18] and no link between this anatomical asymmetry and lateralized behaviors has been reported.[35] These asymmetric structures in the diencephalon are not involved in the lateralized prey-catching behavior in toads, where the stimulus-response is influenced by a telencephalic neuropathway.[36]

Xenopus is the only amphibian in which the cascade of genes leading to visceral asymmetries is described. However, since no directed asymmetries other than visceral and diencephalic are known for this genus, it provides little insight into development of the lateralized behavior and subsequent hemispheric specialization. In other anurans, however, directed asymmetries other than those related to situs viscerum definitely occur.[35]

Linking visceral development and behavior

Some attempts have been made to link the asymmetry of internal organs with overt behaviors in amphibians; most notably right-handedness in toads.[37,38] When toads vomit, they wipe the everted, prolapsed stomach with their right hand. Because of its left-sided position and asymmetric mesenteries, the prolapsed stomach always hangs out the right side of the mouth. Hence the emetic behavior favors right-handedness in anurans. However, this hypothesis is insufficient to explain left-handedness, and lateralized hindlimb use by toads in other actions,[2,18,39] or the perceptual lateralizations in amphibians discussed below. Other hypotheses have been put forward to link specific visceral asymmetries (e.g., spiracle position) with behavioral lateralization in anurans, but no clear connection has been found for either tadpole[40,41] or adult[35] behavior.

This raises the question of how close the links are in vertebrates between the two kinds of left−right asymmetry: situs-related and behavioral. The strongest links may be in birds. At least some lateralized, visually guided behaviors, including those exhibited in pebble-grain discrimination tasks, lateralized attack and copulation, and monocular sleep in chicks[42−44] and pigeons[45] can be experimentally modified by light stimulation during the last days of incubation. Due to embryonic turning, the chick embryo lies in the egg with its right eye closer to the eggshell and its left eye occluded. Thus light may preferentially enter the right eye and subsequently affect the left hemisphere.[42] This asymmetric stimulus implies that, in birds, some lateralized behaviors might be indirectly influenced by embryo rotation in the egg and subsequent asymmetric neural activity in the brain. While in the pigeon asymmetric light stimulation may indeed induce some visual lateralization,[45] in chicks, light probably has only a modulatory effect on the pre-existing brain asymmetries, which are sex, age and imprinting condition dependent[44,46] However this explanation involving light's action in birds is unlikely to apply to amphibians that have relatively small, highly transparent eggs and embryos that orient in various directions within the egg membranes.

Although common genes regulate asymmetry of both visceral organs and some structures in the diencephalon of fish,[30−32] there is no evidence correlating the situs viscerum with motor handedness or perceptual lateralization in humans.[46−49] Genes of the *nodal* cascade are evidently not involved in the development of functional asymmetries in the human telencephalon and there were no clear asymmetry in early gene expression in the human brain.[50] This may imply that development of cerebral hemispheres and subsequent behaviors are not under the control of the same gene cascade as the viscera and structures in the diencephalon. Rather, evidence is accumulating to support epigenetics, driven by steroid hormones, as primary influences on brain hemispheres asymmetry in humans and mice.[48,49]

The development of the viscera and the forebrain is radically different. The majority of situs-related asymmetrical organs develop from endoderm (gut and its derivatives) and mesoderm (heart and blood vessels). Cells from the rostral part of Hensen's node generate the ventral midline endoderm of the foregut, which has been proposed to maintain left−right asymmetry of the heart and ventral (pharyngeal), but not dorsal (neural) head.[51] It is also well known that the pituitary

develops as an endodermal invagination,[27,52] which may influence the development of the rest of the neuroectodermally derived diencephalon.

This model implies that, for example, Broca's area in the telencephalon, which controls speech and is morphologically asymmetric in higher primates,[53] shows no reverse asymmetry in situs inversus individuals. Indeed, reversal of neither handedness (see also Ref. 46, 47) nor language hemispheric dominance was found in three individuals with situs inversus totalis,[54] although reversed asymmetry in the Sylvian sulcus, planum temporale volume (as well as occipital and frontal petalia) was noted. The authors conclude that asymmetry in petalia indeed may be related to that in visceral organs, while other structural and functional asymmetries may not. They further speculate on the possible link between the volume of planum temporale and language lateralization. In another study with a different subject with situs inversus syndrome, reversed anatomic asymmetry was noted also in cranial bones, superficial blood vessels and the relative position of the Sylvian sulcus.[55]

It should be stressed, however, that, although these asymmetries may influence one another, they may not necessary influence the lateralized function of hemispheres. Indeed, although structural and functional asymmetries in the brain may be related,[56] the frequency of language lateralization to the left hemisphere and right-hand preference is much higher than the frequency of asymmetric morphologies.[54]

Interesting, there were no hemispheric reversals noted in twins discordant for writing hand;[50] i.e., only one of 27 monozygotic twin pairs exhibited reversals of Sylvian sulcus morphology. However, the morphology of surrounding perisylvian cortex of one twin was not a reversed image of the other. Unfortunately visceral asymmetries, which may have linked situs asymmetry of visceral organs, brain external morphology and asymmetric brain activity were not studied.

The relative position of hemispheric fissures may merely reflect asymmetry in the braincase and not necessarily a common developmental pathway. Moreover, Sylvian fissure morphology can be affected by perinatal events, showing weak heritability estimates.[50] Unfortunately, no such detailed studies of brain morphology and function were performed on any amphibian species that has both normal and inverse situs viscerum.

In frogs (*Rana "esculenta"*) superficial blood vessels over the telencephalon are distributed mostly asymmetrically, but that was not the case for the deep capillaries.[57] However, vascular asymmetries were neither correlated with nor contribute to the structural or functional asymmetry of the hemispheres. In contrast, cerebral blood flow, which is asymmetric in mammals including humans (see Ref. 58 for discussion), is not strictly linked to any behavioral handedness and may be a result, rather than the cause, of changes in local neuronal activity.[54] For example, high cerebral blood flow was found in the habenulae, but with no significant difference between the right and the left sides.[58]

In sum, currently there are not enough data to confirm that the developmental mechanisms directing asymmetry in the brain's hemispheres and the rest of the body are the same. Although some correlations between the structure and function of the brain hemispheres and visceral asymmetry can exist, they do not necessarily reflect cause-and-effect relationships.

Brain versus viscera asymmetries: head versus trunk evolution?

It was recently proposed that the whole body of cnidarians, which are closely related to the common metazoan ancestor, corresponds to the cephalic portion of the vertebrate and arthropod embryo.[59] The rest of the metazoan body would have then arisen strictly from the zone near the gastric cavity opening in the former cnidarian head. If correct, the origin of bilaterality with the formation of a midline took place very early in metazoan evolution, but only after the emergence of a separate head and trunk and arose independently in arthropods and chordates. Our knowledge of the development of asymmetries fits this proposition.

Unlike vertebrates with pronounced asymmetries in both internal organs and the brain, few asymmetries occur in insects. In *Drosophila*, the most commonly studied insect, there is asymmetric (but random) overlap of wings, an asymmetric spiraling of the copulatory organ and a slight, but biased, helical twisting of the gut. The twist of the gut is reversed in a number of fly mutants.[60,61] However, while some parts of the gut show independent twisting and reversal of handedness,[61] others reveal a maternal (extrinsic) influence on asymmetry.[60] There is an intrinsic chirality or rotational torsion in the *Drosophila* abdomen, which is not visible in wild-type animals, but which can be relaxed in at least four different mutants causing body asymmetry.[62] Collectively, these facts leave the impression that the fruit fly shows more chirality than left–right side differences. Moreover, there are no genes asymmetrically expressed along the left–right axis reported for *Drosophila* or other insects. Taken together, these facts affirm the spiral asymmetry of insects[63] and suggest different causes and regulations for body asymmetries in insects and vertebrates. These differences start with fundamental differences in midline formation between the two groups.[59] Presumably asymmetry of the body in chordates and arthropods evolved independently. However, it is still possible that brain development and behavioral lateralizations have undergone parallel evolution. Indeed, interesting in the context of our review, honeybees are capable of side-specific, non-elemental olfactory discrimination.[64]

In amphibians, birds and mammals, situs-related and head asymmetries (those both associated with behavioral handedness and not) present differently in conjoined and non-

conjoined monozygotic twins.[65,66] Non-conjoined twins do not exhibit the situs viscerum laterality defects that characterize conjoined twins. However, other asymmetries such as hand preference, unilateral eye and ear defects, and other subtle features of the head occur in non-conjoined twins. Interestingly, while the gastrula and early neurula stages are most prone to laterality defects in position of visceral organs, the development of head asymmetry is set earlier—at stages of cleavage, morula and blastocyst, while the unknown initiating event is presumably upstream of both types of asymmetries.[65] Clearly head asymmetries arise developmentally earlier than trunk asymmetries. This corresponds with the probable late evolutionary origin of the chordate trunk, following Meinhardt's "old body to new head" proposition.[59] The expression pattern for genes controling head development evidently became established before the invention of bilaterality. Thus, most probably, development of brain asymmetries has a different genetic and historical background, in contrast to that of most body asymmetries.

If this speculation is true, the separation of visceral and most brain asymmetries likely took place very early in vertebrate evolution and along different regulatory pathways. Indeed, both handednesses in visual control and body asymmetry were characteristics of the earliest chordates.[3,28]

Three "midlines" for one frog?

Vallortigara and Bisazza[3] summarized the current evidence of lateralization in lower vertebrates, discussing it under three headings: anatomical, motor and sensory, stressing that "such subdivisions and terminology are simply based on convenience and do not necessary correspond with any important theoretical issue" (p. 11 in Ref. 3). However, a rationale for this tripartite classification is that lateralizations in each may be regulated independently in amphibian (and more broadly in vertebrate) embryos, i.e. visceral (or anatomical), sensory (perceptual) and motor (locomotor) systems.

Dill found that postmetamorphic tree frogs (*Hyla regilla*), presented frontally with a model predator, showed a significant leftward bias in their escape jumps.[67] Although he discussed this side bias in jumping in context of the frogs' leg length subsequent scientists, who examined direction bias in jumps by three species of toads (*Bufo*), related it to lateralization in a visually guided response to a predator.[12] Specifically they showed visual lateralization of the avoidance response of toads, when a model predator was presented laterally to them. However, they did not find any lateralization in escape jumps, when a model predator was presented frontally. Thus Dill's findings remain unexplained in terms of visual lateralization. We suspect that motor biases, overlapping with eye preference, could affect the results of such experiments. To confirm this, data from more taxa must be collected on motor tasks that are likely not under direct visual control.

In this regard, Bisazza and colleagues tested the forelimb and hindlimb preferences in three species of toads, *B. bufo, B. viridis* and *B. marinus*, by allowing them to use right or left limbs to: (1) remove an object from their snout, or (2) turn upright from the upside-down position underwater or on a horizontal surface in the air.[11,39,68] They found that *B. bufo* and *B. marinus* are consistently right-handed and right-footed species, while *B. viridis* showed left-side preference in foot use and non-significant left preference in the use of forelimbs. These experiments were the first strong indication of limb preference in amphibians and consistent with independent data on eye preferences and hemispheric specialization.[8,19]

A later study confirmed that *B. viridis* is both left-handed and left-footed.[40] More generalized toads of the genus *Bombina* were found to lack these lateralized motor behaviors at the population level, both shortly after metamorphosis (*B. orientalis*[69]), and as adults (*B. bombina*[40]). Other amphibians, more recently studied in the field, were spadefoot toads, *Pelobates fuscus*[36] and green tree frog, *Litoria caerulea*,[2] and they showed no significant population bias in their forelimb use. In contrast, broad-palmed tree frog, *Litoria latopalmata*, revealed a population bias in right forelimb use when they pivot to right themselves from the overturned position.[2]

Focussing only on those genera where both eye preference and motor biases were measured, namely *Bufo* and *Bombina*, we find a uniform lateralization of eye function in both larvae and adults.[10–13] No such uniformity is found with respect to limb use; *Bufo* species appeared to be either left or right handed and footed, while *Bombina* species appeared ambidextrous. Clearly there is no consistent lateralization of perceptual and motor performance across anuran species, although there may occasionally be consistency within a species.

Similarly, in human monozygotic twins discordant for handedness, some pairs had a high degree of resemblance for asymmetry in language-related activity in Broca's area, while other pairs showed opposite patterns of asymmetry.[70] As language dominance is usually correlated with handedness in human singletons, the latter authors suggested that this discordance may be caused by the twinning process itself.[70] This proposition, however, can hardly be proven experimentally. Alternatively, this discordance might indicate not the absolute coordination of all kinds of neurobehavioral asymmetries in development, but rather the result of long independent evolution of brain functions.

Given our knowledge of the development of cerebral asymmetries and lateralized behaviors, it is premature to think that motor and perceptual lateralizations arise by radically different molecular pathways. However, motor and sensory innervation in chick embryos may develop independently.[71] Hypothetically, motor and perceptual lateralizations could evolve independently in vertebrates[36] due to different selective pressures. As lateralization in perception was established

early in evolution,[3] it could later interact and operate differently with the rest of the body, depending on the motor demands of different vertebrates.

Individual asymmetries and populational biases: causes and their regulations

Available data suggest that visceral asymmetry is more conserved within vertebrates than brain and behavior asymmetries; the former being invariant in wild populations. In contrast, behavioral lateralizations are not so stable in populations, being more labile and subject to different selective pressures. This raises the question of distinguishing the symmetry breaking events from the regulatory mechanisms that lateralize the asymmetry (see Refs. 59, 62 for a fuller discussion). To some extent these two successive steps to lateralization may reflect corresponding asymmetries at the individual, versus lateralization at the population, levels.

A theory for the general development of directed asymmetries in animals has been proposed by Brown and Wolpert.[59] It assumes three developmental steps in establishing left–right pattern: conversion, random generation of asymmetry and interpretation. In the first step, a hypothetical handed F-molecule generates a left–right difference between the sides of the embryo via a mechanism that converts the molecular handedness into a cellular one. The second step generates an asymmetry at the cellular or multicellular level, which may be biased by pre-existed handedness (step 1). In the last step, the cellular handedness is "interpreted" into development of particular structures and organs, which emerge on one side of the embryo, but not the other (see Ref. 59 for more details).

Although a candidate F-molecule is not yet identified, it seems unlikely that two or more handed molecules independently regulate the asymmetry of the brain and the viscera—or for that matter, any other asymmetries. Most probably the mechanism that breaks symmetry is common for vertebrates (see Ref. 56 for alternatives in *Drosophila*).

The regulations or random generation of asymmetry may, however, lie outside the Brown and Wolpert model. Given the pronounced differences in the degree of handedness—very high for one asymmetry (situs viscerum) and variable for the others (behavioral lateralization)—one interesting direction for future research will be the search for populational and developmental regulators that lead to this intriguing effect.

The right time for the left turn

The best-examined lateralized behaviors of anurans are the turning biases of their tadpoles (recently reviewed in Ref. 72). Bias in turning can be documented in several ways. Tadpoles, for example, can be threatened or startled; i.e., mechanically, via a shock wave generated when one hits the container they are in,[41] or visually, by bringing an object directly toward their head.[2] Alternatively, bias can be assessed in voluntary turns,

with less overt stress, by scoring tadpoles' turns as they spontaneously exit a tube or turn in a T-maze (for more details see Ref. 72 and references there in, plus other papers in Ref. 6).

Most data, however, have been collected as tadpoles turn to dive, after surfacing to take a breath of air. Tadpoles that break the water's surface are at risk of being picked off by avian predators.[73] Hence it behoves them to make this maneuver quickly. Diving turns are often executed very fast and resemble a startle response, but in a vertical rather than horizontal plane. We believe that such abrupt turns, made under stressful circumstances, are more likely to reliably reveal laterality in behavior and neural processing than more casual turns made under less-stressful circumstances.

The argument that these rapid turns at the water's surfaces are, in fact, equivalent to a startle response is reinforced by observations that the bias intensifies as predator risk increases. *Rana temporaria* tadpoles, for example, which tend to turn to the left 60% of the time after surfacing to breath air, increase that bias to 77% when a screened piscine predator is included in their tank (R.J.W. and Stöttinger, unpublished data).

The way in which turning is provoked seems to yield different results in terms of bias. In test situations where the tadpoles could have made more leisurely turns (i.e., for the ranid frogs *Limnonectes corrugatus* and *Nannophrys ceylonesis* and the hylid *Litoria latopalmata*; Table 1 in Ref. 72) either no bias or left and right biases were observed with about equal frequency. However, in test situations where tadpoles necessarily turned rapidly, such as when they surfacing to breathe air, turning biases were more common and always toward the left (seen in ranids *Rana catesbeiana, Rana sylvatica, Rana temporaria*; hylids *Phrynohyas venulosa, Scinax latopalmata*; and the microhylid *Microhyla ornata*; Table 1 in Ref. 72). Across the board, where turning biases have been observed, they range from approximately 60 to 90% toward the left.

To date, turning bias has been examined in tadpoles from 15 species representing six different families. In the two most generalized (= archeobatrachian) taxa that have been examined so far, *Bombina orientalis* (Bombinatoridae) and *Xenopus laevis* (Pipidae), turning biases were not observed. This led Goree and Wassersug[69] to speculate that turning bias emerged phylogenetically late within the Anura. However, there are some caveats. The *B. orientalis* and *X. laevis* tadpoles that were tested were all relatively mature tadpoles, and both Yamashita et al.[41] and Oseen et al.[74] have presented data to suggest that tadpole turning bias is stage dependent, decreasing as tadpoles approach metamorphosis.

It is also true that older tadpoles usually have been in the laboratory longer and this may lead to diminished startle responses as tadpoles either adapt to, or become depiliated in,

captivity. In the case of tests with both *B. orientalis*[69] and *X. laevis*,[42] the tadpoles were, in fact, laboratory stock maintained from hatching. More archeobatrachian species will need to be examined and preferable of younger stages—and in field situation—before one can say with much certainty how turning bias evolved in anurans.

The absence of a turning bias in *Bombina* and *Bufo* has implications to understanding the neurobiology of turning biases in tadpoles in general. As noted above, the strongest turning biases have been observed in tadpoles when they were startled or turning quickly at the surface. These rapid turns are understood to be mediated by giant motor neurons, called Mauthner cell, which are lacking in *Bombina* and *Bufo* tadpoles.[75,76] [*Bufo* species compensate for this diminished startle response by having toxic tadpoles.[77]]

Wassersug and Yamashita[72] hypothesized that laterized turning in tadpoles is a consequence of asymmetry in the Mauthner's neurons. Such anatomical asymmetry was, in fact, reported in tadpoles.[78] What has not been established is how precisely that anatomical asymmetry matches the behavioral asymmetry: Do tadpoles with the most asymmetric Mauthner neurons also have the strongest turning bias? Although this simple question has not been answered, the presumed correlation of Mauthner cell asymmetry with turning bias constitutes the best evidence that we have linking a specific neural asymmetry in anuran larvae with asymmetrically displayed behaviors.

The adaptive significance of a turning bias in tadpoles *per se* was also discussed by Wassersug and Yamashita.[72] If tadpoles are facing a threat dead ahead, the last thing they would want to do is fire axial muscles equally on both sides of their body. This would simply lock them up. The Mauthner neurons prevent this from happening. By having a "hardwired handedness", a tadpole can turn sharply and quickly to one side, even when a threatening stimulus is sensed equally on both sides of its body.

Wassersug and Yamashita[72] also speculate about other possible asymmetric behaviors in tadpoles. Before hatching anuran embryos coil their tails to one side or the other. This is necessary simply because hatchling tadpoles are longer than the diameter of the eggs from which they emerge. Thibaudeau and Altig[79] suggested, without presented supporting data, that in *Eleutherodactylus coqui* (Leptodactylidae) there was a left-sided bias in terms of the direction in which the tail was held. It would be interesting to know if this bias was statistically significant and common in other anuran embryos.

Before hatching most anuran embryos are active enough to switch their tails from one side to the other...and they do. But it is possible that at an early stage the size of the egg may passively force the tail to one side or the other. A postural "handedness" (which way the tail curls) at those early stages is evidently absent in *Xenopus*,[80] which lacks turning bias. The question remains whether biases in the side toward which the tail is bent before hatching coordinates with, if not directly influences, biases in the motor system that could include the Mauthner neurons themselves. We simply do not know what developmental processes account for the asymmetry in Mauthner neurons.

The most intriguing case of behavioral laterality in amphibian larvae occurs when tadpoles are allowed to view themselves in mirrors. Consistent with the theme that "lower" vertebrate brains are highly lateralized, Bisazza et al.[13] reported that tadpoles from three families and five species of European Anura all position themselves in front of mirrors such that they preferentially view themselves with the monocular field of their left eye. The fact that *Bombina variegata* shows this preference, even though it has a medial spiracle, indicates that this postural bias is not simply coupled to, or driven by, spiracular asymmetry.

A postural bias in mirror viewing has been reported for fish[14] and linked to social behavior in teleosts. Bisazza et al.[13] point out that, at the developmental stages that they examined, their tadpoles had little or no overlapping visual fields. They state that their results "imply that amphibians exhibit a right hemisphere dominance in response to visual stimuli by a conspecfic." This appears to be true both before and after metamorphosis. It should be noted that some of the species Bisazza et al.[13] tested (in the genus *Bufo*) form large dense social schools while others are not so gregarious.

Implicit here is the idea that, if tadpoles are so aware of their own image, they must also be visually aware of conspecifics. This suggests that visual information and not just olfactory clues are used by tadpoles to assess their local density.[81] A multitude of experiments have shown that tadpoles at high density have reduced growth rates (reviewed in Ref. 81). Would tadpoles in aquaria with mirrored sides interpret the plethora of images as if they were at higher density than they really were? Preliminary data show that placing mirrors on the walls of aquaria can in fact reduce tadpole growth rates compared to controls in normal tanks (Rot Nikcevic and R.J.W., unpublished data).

Whether one considers turning bias or posture in front of mirrors, both lateralized behaviors are altered with metamorphosis. In the case of turning bias, the motor hardware changes drastically as the tail is resorbed. The visual system of tadpoles is similarly remodeled to give the frog the overlapping visual fields used to localize prey. Nevertheless the lateralized processing of visual information about conspecifics, which develops well before metamorphosis, is evidently retained.

Crossing over the midline

So far, we have explored possible links in asymmetries of situs viscerum and behavioral handedness in a search for common mechanisms of development. However, there are more asymmetries in amphibians that also show no apparent correlation

to each other or to previously discussed ones,[36] and at least one of them deserves mention.

Much recent attention has been given to epicoracoid overlap in the arciferal shoulder girdle of most amphibian species (see for review[36]). Thin cartilaginous plates of epicoracoids overlap such that either the left dorsally overlaps the right, or the reverse.[82] "Handedness" here does not correlate with either situs viscerum or asymmetries in the length and weights of long bones in limbs (Refs. 36, 83 but see Ref. 40).

Borkhvardt[82] suggested a mechanism that could lead to the overlap of epicoracoid plates. These cartilages, which grow superficially from the base of the limb bud, are so thin in arciferal anurans, that when they meet ventrally on the midline of the body, they have enough room to overlap between the ectoderm and the wall of the thoracovisceral cavity. In contrast, in fermisternal anurans, which have no epicoracoid overlap, the epicoracoids are very thick and take up the whole space between the skin and internal structures (see figures in Ref. 36, 82). However, we still do not know why this asymmetry is lateralized; i.e., why the ratio of overlap pattern (left over right or right over left) differs from random in arciferal species.[36]

There are interesting analogies to epicoracoid overlap asymmetry described in two contralateral neural projections in the goldfish.[84] In the first case, either the left or right optic nerve lies dorsal at the chiasma and originates from a slightly smaller eye. In the second case, the fibre from the left or right Mauthner neuron is dorsal at decussation with the contralateral fibre and originates from the smaller medulla side. This indicates that differential growth rates may be involved in all cases. Indeed, there are some indications that growth rates probably influence epicoracoid overlap,[85] leading us to suggest that, either the developmental rates differ on the two sides or there is asymmetry in the initial position of shoulder girdle

anlage.[36] In the case of the nerves, it is possible that the earlier maturing fibres take the ventral route.[84] In all cases of contralateral structural overlap, it appears developmentally, when the structures are relatively thin and further growth necessitates overlap.

Conclusions

The majority of asymmetries in amphibians fall into two groups (Fig. 2). The first group is asymmetries related to situs viscerum. These are usually invariant in their laterality, with the majority of individuals in a given population having only one of two possible states. Importantly, these asymmetries are directed to the same side in various species, amphibian orders and even vertebrate classes. Thus they appear connected to basic morphological asymmetries, such as those seen in the gut and its derivatives, as well as the vascular system and some brain structures. The genetic background of these asymmetries is fairly well understood.

The second group comprises asymmetries in paired structures whose laterality may not correlate either with one another, or with situs viscerum. Included here are eye and hand preference, and overlap of epicoracoids. For these traits, the degree and direction of lateralization may vary significantly in different populations, species and classes. Genes regulating their development have not yet been identified and heredity of these asymmetries is less studied.

This bipartite classification of asymmetries may be artificial. However, it may also reflect the fundamental differences in underlying developmental mechanisms.

One of the implications of the Brown–Wolpert model[59] for development of laterality is that biasing of the system is one global event (step 1) that takes place early, while generation of random asymmetry (step 2) may involve multiple local
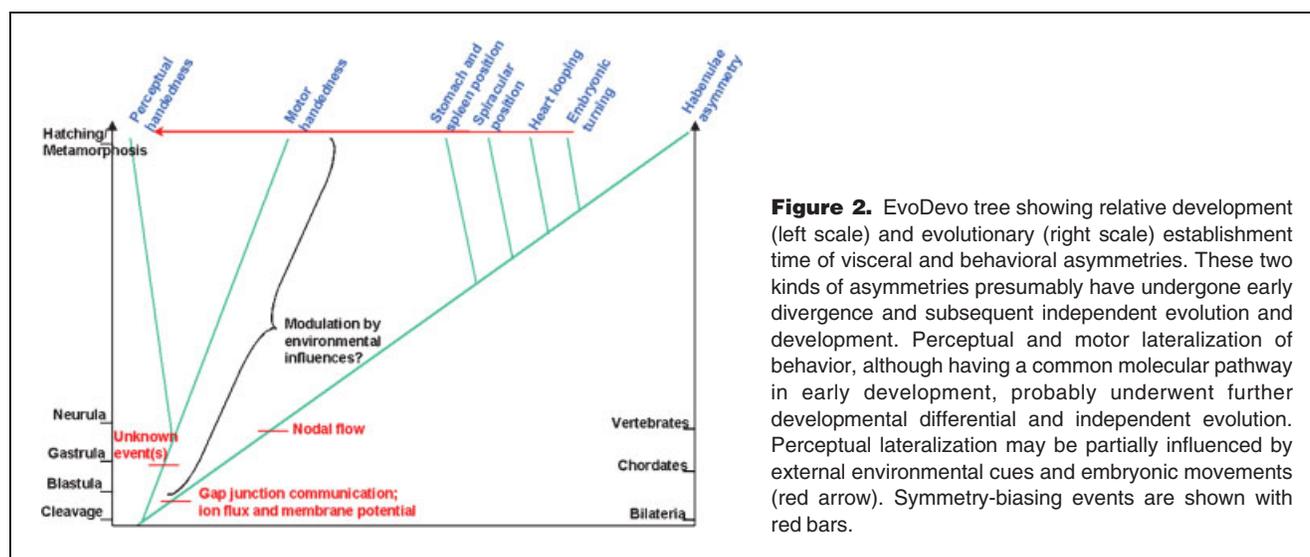
Figure 2. EvoDevo tree showing relative development (left scale) and evolutionary (right scale) establishment time of visceral and behavioral asymmetries. These two kinds of asymmetries presumably have undergone early divergence and subsequent independent evolution and development. Perceptual and motor lateralization of behavior, although having a common molecular pathway in early development, probably underwent further developmental differential and independent evolution. Perceptual lateralization may be partially influenced by external environmental cues and embryonic movements (red arrow). Symmetry-biasing events are shown with red bars.

processes, which take place later in particular organs. In the absence of that biasing event, the generation of random asymmetry appears independent for each structure of the embryo. In animals with a normal biasing system, asymmetries are very highly correlated. This is indeed the case for the asymmetries related to situs viscerum (i.e., asymmetry in heart, gut, spleen, lungs, and operculum) or those controled by the same cascade of genes (i.e. asymmetry in habenulae and parapineal in the diencephalon, embryo rotation). However, for many others, especially functional asymmetries of the brain and behavior, it is not the case.

We have presented examples where asymmetries are most likely randomized at an early developmental step, while biasing seems to be a later event. Many behavioral and some morphological lateralizations in many, if not all, vertebrate groups, are unstable in populations or even absent at the group level, yet always present in individuals. Moreover, when biased, these asymmetries normally do not show a strong, or any, correlation with each other. In some cases, a randomization of almost all asymmetries takes place in certain species.[36] This might result from ancient evolutionary divergence of asymmetric structures and their developmental processes.

We have tried here to review and classify the most recent data on asymmetries in amphibians, comparing them to asymmetries in other vertebrates. After this review was submitted, we learned of a paper by Cooke (in press), which explores many of the same questions that we have.[86] Although based on different observations, Cooke reaches very many of the same conclusions that we have. In contrast, though, Cooke believes that visceral asymmetries appear in evolution much earlier than neurobehavioral ones, which were superimposed on a retained asymmetric "visceral" chordate. We feel that visceral and neurobehavioral asymmetries evolved in parallel, but independently from the earliest steps of chordate evolution. Studies on more taxa beyond the standard model systems (e.g. *Xenopus*) will be essential for selecting between these competing hypotheses and for understanding the evolution of asymmetry in chordates in general.

Acknowledgments

References

1. Bianki VL. 1985. Asymmetry of the animal brain. Leningrad: Nauka; 294 p.
2. Rogers LJ. 2002. Lateralised brain function in anurans: comparison to lateralisation in other vertebrates. Laterality 7:219–239.
3. Vallortigara G, Bisazza A. 2002. How ancient is brain lateralization? In: Rogers LJ, Andrew RJ, editors. Comparative vertebrate lateralization. Cambridge: Cambridge University Press; p 9–69.
4. Spemann H, Falkenberg H. 1919. Über asymmetrische Entwickelung und situs inversus viscerum bei Zwillingen und Doppelbildungen. Arch Entwicklungsmech Org 45:371–422.
5. Rogers LJ, Andrew RJ, editors. 2002. Comparative Vertebrate Lateralization. Cambridge: Cambridge University Press; 660 p.
6. Malashichev YB, Rogers LJ, editors. 2002. Behavioural and Morphological Asymmetries in Amphibians and Reptiles. Special issue. Laterality 7:1–108.
7. Rogers LJ. 2002. Lateralization in vertebrates: its early evolution, general pattern, and development. Advances in the study of behaviour 31:107–161.
8. Andrew RJ, Rogers LJ. 2002. The nature of lateralization in Tetrapoda. In: Rogers LJ, Andrew RJ, editors. Comparative vertebrate lateralization. Cambridge: Cambridge University Press; p 94–125.
9. Vallortigara G, Rogers LJ, Bisazza A, Lippolis G, Robins A. 1998. Complementary right and left hemifield use for predatory and agonistic behaviour in toads. NeuroReport 9:3341–3344.
10. Robins A, Lippolis G, Bisazza A, Vallortigara G, Rogers LJ. 1998. Lateralized agonistic responses and hind-limb use in toads. Animal Behaviour 56:875–881.
11. Lippolis G, Bisazza A, Rogers LJ, Vallortigara G. 2002. Lateralization of predator avoidance responses in three species of toads. Laterality 7:163–183.
12. Bisazza A, De Santi A, Bonso S, Sovrano VA. 2002. Frogs and toads in front of a mirror: lateralization of response to social stimuli in five tadpole amphibians. Behavioural Brain Research 134:417–424.
13. Sovrano VA, Bisazza A, Vallortigara G. 2001. Lateralization of response to social stimuli in fishes: a comparison between different methods and species. Physiology and Behavior 74:237–244.
14. Rogers LJ. 2002. Advantages and disadvantages of lateralization. In: Rogers LJ, Andrew RJ, editors. Comparative vertebrate lateralization. Cambridge: Cambridge University Press; p 126–153.
15. Green A. 1997. Asymmetrical turning during spermatophore transfer in the male smooth newt. Animal Behaviour 54:343–348.
16. Marzona E, Giacomo C. 2002. Display lateralization in the courtship behaviour of the alpine newt (Triturus alpestris). Laterality 7:285–296.
17. Andrew RJ. 2002. The earliest origin and subsequent evolution of lateralization. In: Rogers LJ, Andrew RJ, editors. Comparative Vertebrate Lateralization. Cambridge: Cambridge University Press; p 70–93.
18. Vallortigara G, Rogers LJ, Bisazza A. 1999. Possible evolutionary origins of cognitive brain lateralization. Brain Research Reviews 30:164–175.
19. Ryan AK, et al. 1998. Pitx2 determines left-right asymmetry of internal organs in vertebrates. Nature 394:545–551.
20. McDiarmid RW, Altig R, editors. 1999. Tadpoles: the Biology of Anuran Larvae. Chicago: University of Chicago Press.
21. Huxley JS, De Beer GR. 1934. The Elements of Experimental Embryology. Cambridge: Cambridge University Press.
22. Nonaka S, Shiratori H, Saijoh Y, Hamada H. 2002. Determination of left-right patterning of the mouse embryo by artificial nodal flow. Nature 418:96–99.
23. Essner JJ, Vogan KJ, Wagner MK, Tabin CJ, Yost HJ, Brueckner M. 2002. Conserved function for embryonic nodal cilia. Nature 418:37–38.
24. Levin M. 2003. Motor protein control of ion flux is an early step in embryonic left-right asymmetry. BioEssays 25:1002–1010.
25. Dathe V, Gamel A, Manner J, Brand-Saberi B, Christ B. 2002. Morphological left-right asymmetry of Hensen's node precedes the asymmetric expression of Shh and Fgf-8 in the chick embryo. Anat. Embryol 205:343–354.
26. Levin M, Mercola M. 1999. Gap junction-mediated transfer of left-right patterning signals in the early chick blastoderm is upstream of Shh asymmetry in the node. Development 126:4703–4714.
27. Levin M, Thorlin T, Robinson KR, Nogi T, Mercola M. 2002. Asymmetries in H+/K+-ATPase and cell membrane potentials comprise a very early step in left-right patterning. Cell 111:77–89.
28. Boorman CJ, Shimeld SM. 2002. The evolution of left-right asymmetry in chordates. BioEssays 24:1004–1011.
29. Zhu L, Marvin MJ, Gardiner A, Lassar AB, Mercola M, Stern CD, Levin M. 1999. Cerberus regulates left-right asymmetry of the embryonic head and heart. Curr Biol 9:931–938.

30. Concha ML, Burdine RD, Russell C, Schier AF, Wilson SW. 2000. A Nodal signaling pathway regulates the laterality of neuroanatomical asymmetries in the zebrafish forebrain. Neuron 28:399–409.

31. Long S, Ahmad N, Rebagliati M. 2003. The zebrafish nodal-related gene southpaw is required for visceral and diencephalic left-right asymmetry. Development 130:2303–2316.

32. Gamse JT, Thisse C, Thisse B, Halpern ME. 2003. The parapineal mediates left-right asymmetry in the zebrafish diencephalon. Development 130:1059–1068.

33. Braitenberg V, Kemali N. 1970. Exceptions to bilateral symmetry in the epithalamus of lower vertebrates. J Comp Neurol 138:137–146.

34. Stark S, von Kraft A. 1993. Die Entwicklung der Asymmetrie von Darmtrakt, Herz und Nuclei habenulae des Zwischenhirns beim Bergmolch (Triturus alpestris). Zool Jb Anat 123:103–123.

35. Malashichev YB. 2002. Asymmetries in amphibians: A review of morphology and behaviour. Laterality 7:197–217.

36. Ewert J-P, Buxbaum-Conradi H, Glagow M, Rottgen A, Schurg-Pfeiffer E, Schwippert WW. 1999. Forebrain and midbrain structures involved in prey-catching behaviour of toads: stimulus-response mediating circuits and their modulating loops. Europ J Morphol 37:111–115.

37. Naitoh T, Wassersug RJ. 1996. Why are toads right-handed? Nature 380:30–31.

38. Bisazza A, Cantalupo C, Robins A, Rogers LJ, Vallortigara G. 1996. Right-pawedness in toads. Nature 379:408.

39. Malashichev YB, Nikitina NG. 2002. Preferential limb use in relation to epicoracoid overlap in the shoulder girdle of toads. Laterality 7:1–18.

40. Yamashita M, Naitoh T, Wassersug RJ. 2000. Startle response and turning bias in Microhyla tadpoles. Zoological Science 17:185–189.

41. Wassersug RJ, Naitoh T, Yamashita M. 1999. Turning bias in tadpoles. Journal of Herpetology 33:543–548.

42. Deng C, Rogers LJ. 2002. Factors affecting the development of lateralization in chicks. In: Rogers LJ, Andrew RJ, editors. Comparative Vertebrate Lateralization. Cambridge: Cambridge University Press; p 206–246.

43. Deng C, Rogers LJ. 2002. Pre-hatching visual experience and lateralization in the visual Wulst of the chick. Behavioural Brain Research 134:375–385.

44. Bobbo D, Galvani F, Mascetti GG, Vallortigara G. 2002. Light exposure of the chick embryo influences monocular sleep. Behavioural Brain Research 134:447–466.

45. Skiba M, Diekamp B, Gunturkun O. 2002. Embryonic light stimulation induces different asymmetries in visuoperceptual and visuomotor pathways of pigeons. Behavioural Brain Research 134:149–156.

46. Trostanetzki MM. 1924. Zur Frage uber den situs viscerum inversus totalis. Bull l'Inst Lesshaft 9:7–24.

47. Tanaka S, Kanzaki R, Yoshibayashi M, Kamiya T, Sugishita M. 1999. Dichotic listening in patients with situs inversus: brain asymmetry and situs asymmetry. Neuropsychologia 37:869–874.

48. Roubertoux PL, Le Roy I, Tordjman S, Cherfou A, Migliore-Samour D. 2003. Analysis of quantitative trait loci for bahavioural laterality in mice. Genetics 163:1023–1030.

49. Eckert MA, Leonard CM, Molloy EA, Blumenthal J, Zijdenbos A, Giedd JN. 2002. The epigenesis of planum temporale asymmetry in twins. Cerebral Cortex 12:749–755.

50. Geschwind DH, Miller BL. 2001. Molecular approaches to cerebral laterality: development and neurodegeneration. Am J Med Genet 101:370–381.

51. Kirby ML, Lawson A, Stadt HA, Kumiski DH, Wallis KT, McCraney E, Waldo KL, Li Y-X, Schoenwolf GC. 2003. Hensen's node gives rise to the ventral midline of the foregut: implications for organizing head and heart development. Dev Biol 253:175–188.

52. Gilbert SF. 2000. Developmental Biology. Sunderland: Sinauer Associates, Inc., Publishers; 750 p.

53. Cantalupo C, Hopkins WD. 2001. Asymmetric Broca's area in great apes. Nature 414:505.

54. Kennedy DN, O'Craven KM, Ticho BS, Goldstein MD, Makris N, Henson JW. 1999. Structural and functional brain asymmetries in human situs inversus totalis. Neurology 53:1260–1265.

55. Tubbs RS, Wellons JC, Salter G, Blount JP, Oakes WJ. 2003. Intracranial anatomic asymmetry in situs inversus totalis. Anat Embryol 206:199–202.

56. Geschwind DH, Miller BL, DeCarli C, Carmelli D. 2002. Heritability of lobar brain volumes in twins supports genetic models of cerebral laterality and handedness. Proc Natl Acad Sci USA 101:370–381.

57. Kemali M, Sada E, Fiorino L. 1990. Superficial and deep blood vessel distribution in the frog telencephalon. Reference to morphological brain asymmetries. Z mikrosk-anat Forsch 104:305–315.

58. Kaufman JA, Phillips-Conroy JE, Black KJ, Perlmutter JS. 2003. Asymmetric regional cerebral blood flow in sedated baboons measured by positron emission tomography (PET). Am J Phys Anthropol 121:369–377.

59. Meinhardt H. 2002. The radial-symmetric hydra and the evolution of the bilateral body plan: an old body became a young brain. BioEssays 24:185–191.

60. Ligoxygakis P, Strigini M, Averof M. 2001. Specification of left-right asymmetry in the embryonic gut of Drosophila. Development 128:1171–1174.

61. Hayashi T, Murakami R. 2001. Left-right asymmetry in Drosophila melanogaster gut development. Development Growth and Differentiation 43:239–246.

62. Martin-Blanco E, Garcia-Bellido A. 1996. Mutations in the rotated abdomen locus affect muscle development and reveal an intrinsic asymmetry in Drosophila. Proceedings of the National Academy of Science (USA) 93:6048–6052.

63. Brown NA, Wolpert L. 1990. The development of handedness in left/right asymmetry. Development 109:1–9.

64. Giurfa M. 2003. Cognitive neuroethology: dissecting non-elemental learning in a honeybee brain. Cur Opin Neurobiol 13:726–735.

65. Levin M. 1999. Twinning and embryonic left-right asymmetry. Laterality 4:197–208.

66. Mercola M, Levin M. 2001. Left-right asymmetry determination in vertebrates. Annual Reviews in Cell & Developmental Biology 17:779–805.

67. Dill LM. 1977. 'Handedness' in the pacific tree frog (Hyla regilla). Canadian Journal of Zoology 55:1926–1929.

68. Bisazza A, Cantalupo C, Robins A, Rogers LJ, Vallortigara G. 1997. Pawedness and motor asymmetries in toads. Laterality 2:49–64.

69. Goree B, Wassersug RJ. 2001. Are archeobatrachian anurans ambidextrous?—Assessing handedness in Bombina orientalis. Journal of Herpetology 35:538–541.

70. Sommer IEC, Ramsey NF, Mandle RCW, Kahn RS. 2002. Language lateralization in monozygotic twin pairs concordant and discordant for handedness. Brain 125:2710–2718.

71. Wang G, Scott SA. 1999. Independent development of sensory and motor innervation patterns in embryonic chick hindlimbs. Dev Biol 208:324–336.

72. Wassersug RJ, Yamashita M. 2002. Assessing and interpreting lateralised behaviours in anuran larvae. Laterality 7:241–260.

73. Crump ML, Varia M. 1991. Vulnerability of Pleurodema borelli tadpoles to an avian predator: Effect of body size and density. Herpetologica 47:316–321.

74. Oseen K, Newhook LKD, Wassersug RJ. 2001. Turning bias in woodfrog (Rana sylvatica) tadpoles. Herpetologica 57:432–437.

75. Will U. 1986. Mauthner neurons survive metamorphosis in anurans: A comparative HRP study on the cytoarchitecture of Mauthner neurons in amphibians. The Journal of Comparative Neurology 244:111–120.

76. Will U. 1991. Amphibian Mauthner cells. Brain, Behaviour, and Evolution 37:317–332.

77. Wassersug RJ. 1973. Aspects of social behavior of anuran larvae. In: Vial JL, editor. Evolutionary Biology of the Anurans: Contemporary research on major problems. University Columbia: Missouri Press; p 273–297.

78. Moulton JM, Jurand A, Fox H. 1968. A cytological study of Mauthner's cells in Xenopus laevis and Rana temporaria during metamorphosis. Journal of Embryology and Experimental Morphology 19:415–431.

79. Thibaudeau G, Altig R. 1999. Endotrophic anurans: Development and evolution. In: McDiarmid RM, Altig R, editors. Tadpoles: The Biology of Anuran Larvae. Chicago: University of Chicago Press; p 170–188.

80. Levin M. 2004. Left-Right Asymmetry in Amphibian Embryogenesis. In: Heatwole H, editor. Amphibian Biology. Volume 6. Embryology; in press.

81. Alford RA. 1999. Ecology: Resource Use, Competition, and Predation. In: McDiarmid RM, Altig R, editors. Tadpoles: The Biology of Anuran Larvae. Chicago: University of Chicago Press; p 240–278.

82. Borkhvardt VG. 1993. Development of arciferal and firmisternal connections between the pectoral girdle halves in amphibians. Zoologicheskiy Zhournal 72:74–84.

83. Robins A, Rogers LJ. 2002. Limb preference and skeletal asymmetry in the cane toad, Bufo marinus (Anura: Bufonidae). Laterality 7:261–275.

84. Andrew RJ. 2002. Behavioural development and lateralization. In: Rogers LJ, Andrew RJ, editors. Comparative Vertebrate Lateralization. Cambridge: Cambridge University Press; p 157–205.

85. Borkhvardt VG, Malashichev YB. 2001. Epicoracoid overlap in fire-bellied toads, Bombina bombina from parents of known morphology. Amphibia-Reptilia 22:480–484.

86. Cooke J. 2004. Developmental mechanisms and evolutionary origin of vertebrate left/right asymmetries. Biol Rev Cambridge Philosoph Soc, in press.