Symmetry Breaking and the Evolution of Development

A. Richard Palmer

Because of its simplicity, the binary-switch nature of left-right asymmetry permits meaningful comparisons among many different organisms. Phylogenetic analyses of asymmetry variation, inheritance, and molecular mechanisms reveal unexpected insights into how development evolves. First, directional asymmetry, an evolutionary novelty, arose from nonheritable origins almost as often as from mutations, implying that genetic assimilation ("phenotype precedes genotype") is a common mode of evolution. Second, the molecular pathway directing hearts leftward—the nodal cascade—varies considerably among vertebrates (homology of form does not require homology of development) and was possibly co-opted from a preexisting asymmetrical chordate organ system. Finally, declining frequencies of spontaneous asymmetry reversal throughout vertebrate evolution suggest that heart development has become more canalized.

Mushrooming information on molecular mechanisms of development, coupled with increasingly robust phylogenetic trees, offers potentially revolutionary insights into how development evolves (*1*–*3*). But general hypotheses remain hard to test because organisms differ so much in form. Evolutionary novelties pose an acute problem because comparable traits, such as wings or image-forming eyes, have emerged too few times independently to allow multiple tests. Informative insights should come most readily from traits that (i) are well defined and unburdened by troublesome semantics, (ii) are easy to compare anatomically and developmentally, and (iii) have evolved multiple times independently. Bilateral asymmetries meet these criteria nicely.

The Binary Asymmetry Switch

Left-right asymmetry offers a particularly attractive focus for comparative studies because of its binary-switch nature. This simplicity follows naturally from the arrangement of developmental axes.

The coordinate system that provides positional information to developing organisms has a property that is often unappreciated: It consists of four axes, not three. Although the anteroposterior and dorsoventral axes, which define the midplane, are both single axes, no single "left-right axis" exists because no single gradient extends from left to right. Rather, "left" and "right" axes are separate mediolateral axes that originate at and extend in opposite directions away from the midplane (*4*). Because these two mediolateral axes are mirror images, an extra symmetry-breaking step must occur for one to differ from the other (*5*). This implies bilateral symmetry is a default state once the anteroposterior and dorsoventral axes are defined, just as radial symmetry is the default state when only one axis exists. So, for example, in a bilaterally symmetrical organism, only one program is needed to specify a limb (*2*), but it yields paired, symmetrical limbs because additional information is required to prevent it.

Left-right differences, therefore, arise because some kind of switch causes the mediolateral axis on one side to differ from the axis on the other side (*4*), although the mechanisms remain unclear for most organisms. Both the origin of this simple binary switch and the subsequent evolution of developmental systems underlying it may be readily compared among taxa of widely differing form. Furthermore, conspicuous asymmetries have evolved independently in many animals and plants (*6*–*8*), so far-reaching generalizations are possible.

Conspicuous Bilateral Asymmetries and Their Inheritance

The bewildering variety of conspicuous morphological asymmetries (*6*–*8*) might seem to defy simple categorization. For example, left and right members of a bilateral pair may differ on otherwise symmetrical individuals, such as the claws of male fiddler crabs (Fig. 1) and many other decapods, the anteriorly directed incisors of narwhals, or the ear openings of some owls. Alternatively, a solitary medial structure may deflect, or rotate, to one side, such as the bill of crossbill finches, the mouth of some scale-eating cichlid fishes, or the twisted abdomen of many male insects. Finally, the entire body may be asymmetrical, as in lopsided verrucomorph barnacles, flatfishes, and animals with coiled shells like snails and spirorbid polychaetes (*7*).

However, emerging from this diversity are two fundamentally different, yet easily distinguished, types (*8*): antisymmetry, in which dextral and sinistral forms are equally common within a species, and directional asymmetry, in which most individuals are asymmetrical in the same direction. These two asymmetry types differ in an important way. In antisymmetric species, direction of asymmetry is almost never inherited, whereas in directionally asymmetric species, it typically is.

It seems remarkable that such conspicuous phenotypes as dextral and sinistral should not be inherited. Yet in virtually all 29 cases of plant and animal antisymmetry, dextral and sinistral offspring were equally frequent regardless of parental phenotype (table S1, section a). In the only compelling exception, alternate alleles control the direction of style bending in enantiostylous flowers of one *Heteranthera* species (*9*).

In contrast, the direction of asymmetry typically is inherited when mutations reverse the orientation of directional asymmetry (table S1, section b), and Mendelian inheritance predominates (seven of nine cases). However, mutations in directionally asymmetric species may have two other phenotypic effects. First, they may randomize direction of asymmetry (i.e., yield antisymmetry), which suggests that they direct the binary asymmetry switch but are irrelevant to subsequent organogenesis [e.g., heart asymmetry in mice (16 genes) and zebrafish (13 genes); brain, liver, and pancreas asymmetry in zebrafish (9 genes); and embryonic cell movements in *Caenorhabditis elegans* (1 gene) (table S1, section c)]. Second, mutants may be symmetrical, which implies that these genes facilitate interpretation (*5*) of left-right differences [19 genes in mice and zebrafish (table S1, section d)]. This

Systematics and Evolution Group, Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada, and Bamfield Marine Sciences Centre, Bamfield, BC V0R 1B0, Canada. E-mail: rich.palmer@ualberta.ca

critical difference in how direction of asymmetry is inherited permits a powerful test of two competing hypotheses about the evolutionary origin of novel forms.

Symmetry Breaking and the Evolutionary Origin of Novel Forms

For more than a century, evolutionary biologists have fretted about how novel phenotypic variation originates (*10*, *11*). Such variation is the raw material upon which natural selection acts to yield morphological diversity and is, therefore, central to all models of evolutionary change. The alternatives seem disarmingly simple: Both mutations and effects of growth environment may generate novel forms. Although few biologists question the evolutionary importance of mutation, debate continues over whether ecophenotypic responses represent simple microevolutionary fine tuning or an important source of new variation (*11*, *12*).

These alternate sources of new variation underlie two fundamentally different modes of evolution (*11*). In the classical neo-Darwinian genotype-precedes-phenotype mode, mutations initially generate more extreme forms. In the unconventional phenotype-precedes-genotype mode, sometimes called genetic assimilation (*13*), developmental plasticity creates novel phenotypes before heritable variation that affects their development. This ultimately arises later by means of random mutations (*14*). Distinguishing these alternate modes is not easy (*14*, *15*), because, as Simpson noted long ago, ''…when the characters in question are demonstrated to be hereditary, there is no evidence whatever that they had occurred as [plastic responses] before they became hereditary'' [p. 113 of (*16*)]. This ''ghost of ecophenotypic variation past'' (Fig. 1) is frustratingly elusive.

Prevalence of genetic assimilation. Fortunately, conspicuous asymmetries allow the prevalence of these alternate modes of evolution to be quantified. Because the direction of asymmetry is not inherited in antisymmetric species (table S1, section a), whereas it is inherited in directionally asymmetric ones (table S1, section b), two alternate routes to directional asymmetry (routes a and b, Fig. 1) correspond to these alternate modes of evolution. The second leg of route b captures the essence of genetic assimilation, in which conspicuous phenotypic variation occurs before genetic variation exists to control it (*11*, *14*).

Evolutionary transitions among asymmetry states may be inferred with standard cladistic methods (Fig. 2). An extensive survey of such transitions yields two important conclusions. First, independent transitions from symmetry to antisymmetry were nearly as common as from symmetry to directional asymmetry (Fig. 1). Indeed, initial asymmetry variation appears to have arisen as often from nongenetic causes as from mutations affecting the direction of asymmetry. Second, directional asymmetry arose from antisymmetry in 36% (16 of 44) to 44% (28 of 63) of cases, so the evolutionary novelty ''directional asymmetry'' appears to have evolved through genetic assimilation (route b, Fig. 1) almost as frequently as through conventional evolution (route a, Fig. 1). Because these inferences necessarily underestimate its prevalence (Fig. 1), genetic assimilation of asymmetry is likely even more common than these numbers suggest.

Ironically, even the sole case where direction of asymmetry is inherited in an antisymmetric species [floral enantiostyly (table S1, section a)] represents a compelling example of genetic assimilation. Among enantiostylous species, the inherited form of floral asymmetry (in which styles of all flowers on an individual plant bend the same way) is not only rare but derived, perhaps twice, from the more common situation in which the direction of asymmetry is not inherited (i.e., dextral and sinistral flowers are equally common on one plant and therefore the direction of asymmetry in an individual flower is, by definition, not inherited) (*17*). So asymmetrical floral phenotypes evolved before mutations that eventually controlled the direction of style bending.

Every taxon that possesses both antisymmetric and directionally asymmetric species, no matter what the trait, offers an independent test for genetic assimilation: Each case in which antisymmetry precedes directional asymmetry evolutionarily (Fig. 2) represents another example. More studies of such taxa would be welcome.

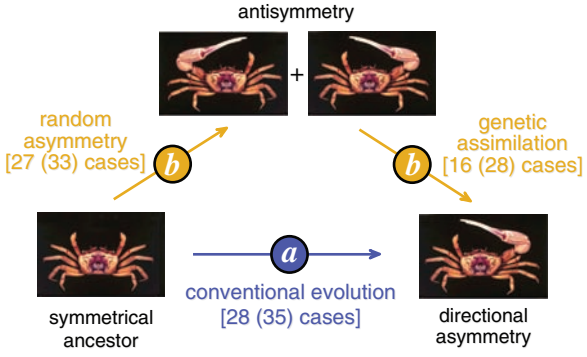

Fig. 1. Two evolutionary routes to directional asymmetry. (Route a) Conventional evolution (genotype precedes phenotype). Initially, a mutation induces asymmetry in a particular direction. Before fixation, two phenotypes coexist: symmetric and dextral or symmetric and sinistral. (Route b) Genetic assimilation (phenotype precedes genotype). Initially, a mutation induces asymmetry without directional bias. Before fixation, three phenotypes coexist: dextral and sinistral (individuals carrying the mutation) and symmetric (individuals lacking the mutation). Upon fixation, dextral and sinistral phenotypes persist in equal frequencies (antisymmetry). Along the second (right) leg of route b, an additional mutation arises that biases asymmetry in one direction. Before fixation, dextral and sinistral phenotypes coexist but one is more abundant (e.g., for a dominant dextral mutation, dextral individuals include antisymmetric and dextral genotypes, whereas sinistral individuals represent only the antisymmetric genotype). The second leg of route b corresponds to genetic assimilation because a conspicuous phenotype (e.g., dextral) that is not heritable is replaced evolutionarily by one that is. Notably, if no antisymmetric species occur within a clade, either living or as fossils, then genetic assimilation cannot be inferred. Such evolutionarily ephemeral, undetected antisymmetry yields "ghosts of genetic assimilation past" (*14*) and may be much more common than currently believed (*15*). Numbers of evolutionary transitions compiled from table 3 in (*7*). Numbers outside parentheses include only reliably inferred phylogenetic transitions; numbers inside parentheses include all inferred transitions regardless of reliability.

Evolution of Developmental Pathways: Vertebrate Heart Asymmetry

All living vertebrates possess a heart that is conspicuously asymmetrical and normally displaced toward the left (*18*). Remarkable progress has been made unraveling the molecular mechanisms directing this asymmetry (*19*–*23*). Variation in the nodal signaling cascade, in which the transforming growth factor–β (TGFβ) family member Nodal plays a central role, paints a particularly clear picture of how the molecular control of development evolves.

Formal phylogenetic analyses of the nodal cascade among vertebrates and protochordates allow tests of two emerging hypotheses about the evolution of development: (i) Developmental system drift [in which ''developmental pathways … diverge through time, even with no accompanying change in [form]'' (*24*) such that homologous structures do not have homologous development (*25*)] and (ii) gene recruitment or cascade capture [in which ancestral gene functions (*3*) or a cascade of several interacting genes (*2*, *26*) are conserved but express or act differently in different tissues or developmental stages].

Developmental Drift: Homology of Form Versus Homology of Development

Although many developmental biologists emphasize the conserved elements of the nodal cascade underlying vertebrate heart asymmetry, others note the many surprising differ-

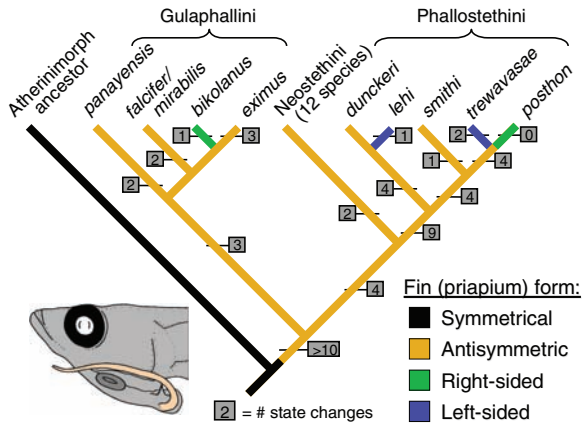

Fig. 2. Evolutionary history of asymmetry in the priapium, a hypertrophied fin modified as an intromittent organ (colored structure, inset) in male phallostethid fish (*70*), reconstructed by means of standard phylogenetic methods (*79*). The ancestral state was clearly antisymmetric, and four species independently evolved right or left sidedness (directional asymmetry). Numbers inside boxes indicate the number of morphological changes supporting each branch (*70*).

ences (supporting online material text). These divergent emphases reveal how disparate expectations are about the evolution of development. Earlier reviews discuss both expression patterns and molecular mechanisms, but to keep the broad comparisons attempted here manageable, I will focus primarily on expression patterns. Presumably, a gene expressed asymmetrically at a particular place and time in one organism, but not in another, implies different molecular mechanisms.

Genes directing vertebrate heart asymmetry: The nodal signaling cascade. Similar to the sometimes idiosyncratic molecular mechanisms that define the anteroposterior and dorsoventral axes (*27*, *28*), genes of the vertebrate nodal cascade include a curious mix of conserved and divergent elements (Fig. 3A). Four highly conserved genes that define the core of the nodal cascade in all vertebrates examined so far—*Nodal*, *Lefty1*, *Lefty2*, *Pitx2* (for family membership and functions of all genes mentioned, see table S2)—are all expressed asymmetrically near the midline or in the lateral plate mesoderm at comparable developmental stages.

Nonconserved elements of the nodal cascade, however, greatly outnumber conserved ones (Fig. 3A). Downstream from the conserved core, four genes differ. Two expressing on the left in other vertebrates express on the right in mice: *Nkx3.2* and *Dante*. Another, *BMP4*, expresses on the left in zebrafish and *Xenopus* but symmetrically in chickens and mice. Finally, *Snail*/*SnR* shows the opposite pattern: right-sided expression in birds and mice but symmetrical expression in zebrafish and *Xenopus*.

Upstream differences seem bewildering (Fig. 3A). First, in chickens, 15 genes/proteins act asymmetrically upstream of *Nodal*: *Fgf4*, *Fgf8*, *Fgf18*, *BMP4*, *Activinβb*, *ActRIIa*, *Chordin*, *Noggin*, *CFC1*, *Shh*, *Hnf3β*, *Lunatic fringe*, *Delta-like1*, an H+/K+ ATPase, and an epithelial structural protein (*N-cadherin*) that functions only on the right. Furthermore, except for *BMP4* (table S2), all but one (H+/K+ ATPase) express symmetrically in other model vertebrates, although information is unavailable for some. Second, in *Xenopus*, three additional asymmetries occur: right-sided persistence of maternal H+/K+ ATPase mRNA in the two- and four-cell embryo, right-sided phosphorylation of Syndecan-2 in the gastrula, and left-sided processing of Vg1 preprotein in the blastula. Here again, where information is available, these are either not present or symmetrical in zebrafish, chickens, and mice (except for H+/K+ ATPase; table S2). Even early perinodal expression of *Nodal* is not entirely conserved, occurring on the left in birds and mammals but not *Xenopus* (Fig. 3A). Furthermore, a different nodal-related gene (*Spaw*) expresses perinodally in zebrafish (*29*).

Reassuringly, patterns in quail (*30*) parallel those in chickens (Fig. 3A) as patterns in rabbits parallel those in mice [although FGF8 function in rabbits is more similar to chickens than mice (*31*)]. These similarities show that more closely related species do appear to have more similar molecular mechanisms orienting heart asymmetry.

Variation in the nodal cascade among vertebrates, like variation in mechanisms controlling primary axis formation (*28*), resembles an hourglass (*32*, *33*): a short, conserved core in the middle of the signaling pathway, the Nodal-Lefty1-Lefty2-Pitx2 pathway, with divergent elements upstream and downstream (Fig. 3A). Vertebrate embryos, therefore, appear to pass through a molecular phylotypic stage, a conserved stage that may provide clues about ancestral developmental pathways, during ontogeny, just as they appear to do morphologically (*34*).

Above all, one conclusion is inescapable: The molecular pathway underlying the binary asymmetry switch that yields a left-sided heart, an unambiguously homologous state in all vertebrates, includes more divergent than conserved elements. Developmental system drift (*24*) may therefore be the rule rather than the exception as development evolves (*28*).

Role of node monocilia in symmetry breaking. Controversy surrounds the role of node monocilia in orienting heart asymmetry (*21*, *35*–*37*). In mice, they normally generate a leftward flow across the node (*38*). But in *iv* mutants, where a defective left-right dynien stalls the molecular motors that propel monocilia (*39*), this leftward flow disappears (*38*) and heart orientation is randomized (*39*). Furthermore, experimentally manipulated flow can induce *Nodal* expression on either side of the node, depending on flow direction (*40*). These results have a powerful theoretical appeal (*21*, *37*). They permit directional asymmetries at the molecular level—chiral arrangement of dynein motor proteins around the central axis of a monocilium (*41*)—to orient directional asymmetries at the morphological level (*5*). They also raised hopes that the holy grail of vertebrate left-right asymmetry studies was near: Could ciliary-driven nodal flow be the universal symmetry-breaking event (*21*, *35*)?

Unfortunately, the story for nonmammalian vertebrates remains unclear. Node monocilia do occur in chickens, *Xenopus*, and zebrafish and do appear before asymmetrical *Nodal* expression (*35*). But problematic observations challenge their symmetry-breaking role. In chickens, node cells are arranged inappropriately when monocilia develop (*42*), and five molecular asymmetries occur before node monocilia fully form (*Hnf3β*/*FoxA*, *Noggin*, *Activin*, N-cadherin, and H+/K+ ATPase; Fig. 3A). In *Xenopus*, four other molecular asymmetries (phosphorylated Syndecan-2, processed Vg1 protein, *BMP4*, and maternal H+/K+-ATPase mRNA) also occur before node monocilia appear (Fig. 3A), and perinodal *nodal* expression, the first consequence of nodal flow, is symmetrical (*43*). Finally, in zebrafish, although the ciliated Kupfer's vesicle plays a critical role in left-right asymmetry (*44*), cilia hydrodynamics was not separated from other effectors.

Fortunately, four critical tests are possible. If node monocilia initiate symmetry breaking, then inactivation by mutation or morpholino knockdown of any of four genes that, in mice, affect cilia formation or function (*45*) should randomize heart asymmetry in other vertebrates. These include (i) the molecular motor left-right dynein, which propels monocilia motion, (ii) Polaris proteins, which aid intraflagellar transport and monocilia assembly, and (iii) KIF3A and KIF3B proteins, kinesins required for ciliary axoneme formation. If inactivation of any one of these fails to randomize heart asymmetry, ciliary-driven nodal flow would be rejected as the conserved symmetry-breaking step.

Gene Recruitment and Cascade Capture

Some key nodal cascade genes have also been studied in lower chordates (*46*), thereby allowing the evolutionary history of gene-

expression patterns and anatomical asymmetries to be compared. More than one surprise emerges.

First, two core members, related to *Nodal* and *Pitx2*, exhibit comparable left-sided expression in some larvae from the two remaining chordate subphyla: ascidians (Urochordata) and lancelets (Cephalochordata) (Fig. 3A). Thus, asymmetrical expression of nodal cascade elements may have been an ancient chordate characteristic. *Nodal* expression in lancelets is markedly similar to vertebrates (*47*). Unfortunately, easy comparisons end here. Although expressed asymmetrically at comparable ontogenetic stages, *Nodal* (*48*) and *Pitx* (*49*) express epidermally in ascidian larvae, and *Pitx* expresses in all three germ layers of both lancelets examined (*49*). This differs from vertebrates, in which they express only in paraxial mesoderm. Furthermore, ascidians, lancelets, and lampreys possess only one *Pitx* gene, versus two in vertebrates, and functional similarities remain unclear (*46*).

The second surprise involves *Shh*, a key nodal cascade gene in birds. *Shh* also expresses (as *AmphiHh*) on the left of lancelet larvae (Fig. 3A). But, here again, differences with vertebrates outnumber similarities. In lancelets, *AmphiHh* expresses in pharyngeal endoderm versus paraxial mesoderm, and it follows rather than precedes asymmetric *Nodal* expression, suggesting a wholly different function (*47*).

What anatomical asymmetry did these nodal cascade genes guide originally? Many internal organs are asymmetrical in vertebrates, including the heart, liver, lungs (in tetrapods), stomach, and brain (Fig. 3B). The heart exhibits the earliest asymmetry ontogenetically (*50*) and has been advanced as the nodal cascade's ancestral target, though the gut has also been proposed (*46*). However, lancelets possess multiple contractile vessels rather than a discrete heart and only the anteriormost aortic arch is obviously asymmetrical (*51*), perhaps as a byproduct of the peculiarly asymmetrical development of lancelet larvae (*52*). Similarly, where one occurs, the ascidian larval heart is also symmetrical (*53*), although it does become asymmetrical during metamorphosis into a sessile adult (*52*). Therefore, in ascidian and lancelet larvae, the heart is presumably not the target of asymmetrically expressed genes.

Brain asymmetry seems a more likely ancestral target. First, some vertebrate brain regions are consistently asymmetrical [most notably, the size differs in the habenulae of fishes and amphibians (Fig. 3B)], and these arise early ontogenetically (*54*). Second, the early onset of heart asymmetry may actually be a derived state in vertebrates, because it occurs progressively earlier throughout amniote evolution (*55*). Third, *Nodal*, *Lefty1*, and *Pitx2* all express asymmetrically during zebrafish brain development (*56*), so key elements of the nodal cascade also orient brain asymmetry [information is lacking for other vertebrates (*56*)]. Finally, ascidian (*54*) and lancelet (*57*) larvae also exhibit conspicuous brain asymmetries, and these appear ontogenetically before asymmetries in the gut or heart (*51*, *53*).

If the nodal cascade primitively regulated brain asymmetry, then its role orienting heart asymmetry is secondary. Furthermore, the direction of heart and brain asymmetry is often discordant in mutant zebrafish (*56*) and in humans with *situs inversus* (*58*), suggesting that no single pathway controls it. Therefore, vertebrate heart asymmetry may represent another case of cascade capture (*3*) during the evolution of development.

Finally, the many genes expressed upstream of asymmetric *Nodal* expression in birds (Fig. 3A) suggest another case of

A) Asymmetrical expression or effect during larval development

Gene or trait	*Side of expression or function*	*Location*	(larval) Ascidian	Lancelet	Zebrafish	Xenopus	Quail	Chick	Mouse	Rabbit
BMP4	left	heart tube	?	sym*	yes	yes	?	sym	sym	?
Nkx3.2	left	LPM	?	?	?	yes	?	yes	right	?
Pitx2	left	LPM	yes*	yes*	yes	yes	yes	yes	yes	yes
Snail/SnR	right	LPM	sym	sym	sym	sym	yes	yes	yes	?
Lefty2	left	LPM	?	?	yes	yes*	?	yes	yes	yes
Nodal	left	LPM	yes*	yes	yes	yes	yes	yes	yes	yes
Caronte/Cerberus	left	paraxial/LPM	?	?	sym	sym	yes	yes	right	?
Lefty1/Antivin	left	perinodal/paraxial	?	?	yes	yes*	yes	yes	yes	yes
Nodal	left	perinodal/paraxial	?	yes	yes*	no expr	yes	yes	yes	yes
Ciliary 'nodal flow'	n/a	node/organizer	?	?	?	no	?	no	yes	?
Node monocilia	midline	node/organizer	?	?	yes	yes*	?	yes*	yes	?
Lunatic fringe	left	perinodal/paraxial	?	sym	sym	sym	?	yes	sym	?
Cryptic/CFC1/oep	left	perinodal/paraxial	?	?	sym	sym*	?	yes	sym	?
Fgf8	right	perinodal/paraxial	sym	?	sym	sym	yes	yes	sym	sym
BMP4	right	perinodal/paraxial	sym*	sym*	sym*	yes*	?	yes	sym*	sym
Shh	left	perinodal/paraxial	sym	yes*	sym	sym	yes	yes	sym	sym
Delta-like1	left	perinodal/paraxial	?	?	sym	?	?	yes	sym	?
Chordin	left	perinodal/paraxial	sym*	?	sym*	sym*	?	yes	sym	?
ActRIIA	right	perinodal/paraxial	?	?	sym	?	yes	yes	sym	sym
Fgf18	right	perinodal/paraxial	?	?	?	?	?	yes	sym	?
Hnf3 β	left	perinodal/paraxial	sym	right*	sym	?	?	yes	sym*	sym
Noggin	right	perinodal/paraxial	?	?	sym*	sym*	?	yes	sym	?
Fgf4	right	perinodal/paraxial	?	?	no expr	sym	?	yes	sym	?
Activin β b	right	perinodal/paraxial	?	?	sym	sym	?	yes	sym	sym
N-cadherin	post-L/ant-R	primitive streak	?	?	?	?	?	yes	?	?
phosph.Syndecan-2	right	side of gastrula	sym*	?	?	yes	?	?	?	?
matureVg1 or gdF1	left	side of blastula	?	?	no expr	yes	?	sym*	sym*	?
H+/K+ ATPase	right	by 2-cell stage	?	?	?	yes	?	yes*	no*	?
calcium signaling	side of polar	body, 2-cell stage	yes	?	?	?	?	?	?	?

B) **Anatomical asymmetries (in larvae)**

Trait								
gut branched, coiled, or looped to one side	yes?*	yes	yes	yes	yes	yes	yes	yes
heart displaced to left of midline	no?*	no	yes	yes	yes	yes	yes	yes
brain/anterior CNS (habenular nucleus in vertebrates)	yes	yes	yes(L)	yes(L)*	?	yes(L)*	yes(R)*	?

Fig. 3. (**A**) Genes expressed or functioning asymmetrically during early development and (**B**) the presence of selected anatomical asymmetries and phylogenetic relations among deuterostome taxa. Each colored column applies to one taxon. Each row applies to a single gene or trait. Colored entries indicate asymmetrical expression or function as indicated in column 2. Noncolored entries mean expression or function differed from that indicated in column 2. Gene order from bottom to top parallels temporal order of expression as closely as possible. LPM, lateral plate mesoderm; sym, symmetrical expression; no expr., no expression; ?, no data or not known; n/a, not applicable; L, left; R, right; perinodal/paraxial, on the periphery of or adjacent to the node or its equivalent in amphibians, fish, and lower deuterostomes; CNS, central nervous system; *, table entry requires clarification or expression order differs from that in the figure. For full gene names, references, information on temporal order of expression, and notes and caveats, see table S2.

cascade capture. The *Noggin*/*Chordin* regulation of *BMP4*, which guides vertebrate-dorsoventral axis formation (*59*), may have acquired double duty controlling left-sided *Nodal* expression, much like genes controlling primary body axes have been co-opted to control limb outgrowth along the mediolateral axes (*26*). Gene recruitment or cascade capture may be the rule rather than the exception as development evolves.

Evolution of Canalization

The notion of evolutionary "progress" remains controversial (*60*). Here, too, a comparative study of symmetry breaking permits a quantitative test: Does development of an established trait, such as the direction of asymmetry, become more canalized (*61*)—i.e., resistant to both genetic and environmental perturbations (*62*)—over evolutionary time?

Although left-sided heart asymmetry is conserved in vertebrates (*18*), occasional reversed individuals do occur. Remarkably, the incidence of spontaneous reversal appears to have declined throughout vertebrate evolution (Fig. 4), from about 5% in fish, to 1 to 2% in amphibians and birds, and notably to less than 0.1% in mammals, including a solid estimate for humans of about 0.01%. This trend suggests an evolutionary increase in canalization.

Whether increased canalization results from different developmental mechanisms or environmental predictability during development remains unclear. The order-of-magnitude decline in spontaneous asymmetry reversals in mammals might result either from increased predictability of symmetry breaking when controlled by cilia-driven nodal flow (*63*) or from more predictable conditions experienced during placental environment. Comparisons between egg-laying and viviparous species of any vertebrate group would provide informative tests. If live bearers exhibit fewer spontaneous reversals, then embryonic environment may influence canalization more than molecular mechanisms of development.

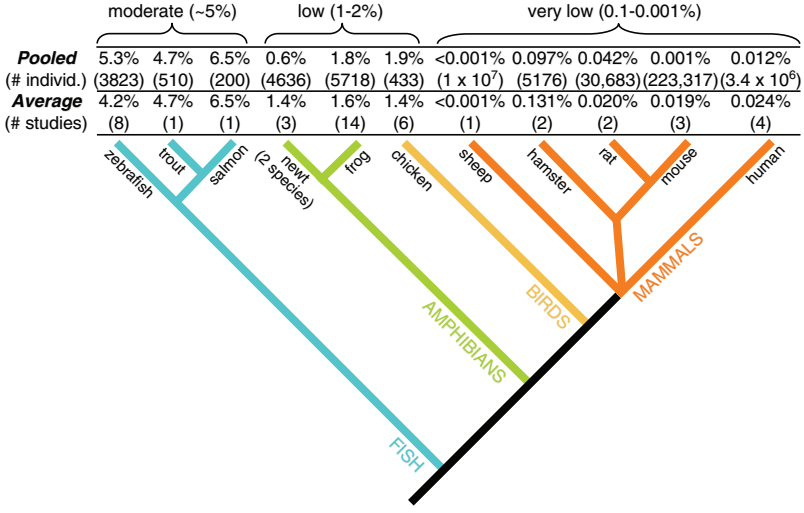

Fig. 4. Incidence of spontaneous reversal of heart asymmetry in vertebrates. Pooled percentages were computed by summing raw counts before computing a percentage. Average percentages were computed as a simple average of percentages reported in each study, regardless of sample size. For references and notes, see table S3.

Loose Ends

Ancestral asymmetric gene-expression pattern in vertebrates. Which expression pattern upstream of *Nodal* is primitive: that in *Xenopus* and chickens (multiple asymmetric genes) or the one in zebrafish and mice (no asymmetric genes) (Fig. 3A)? More studies in zebrafish and nonmodel vertebrates (particularly a lamprey or squamate reptile) would help. If multiple genes typically express asymmetrically upstream of *Nodal* in nonmammalian vertebrates, then cilia-driven symmetry breaking may be unique to mammals and might contribute to their notably higher canalization of left-right asymmetry (Fig. 4), because normal heart orientation would require fewer potentially vulnerable upstream genes.

Developmental basis of antisymmetry. Direction of asymmetry is almost never inherited in species exhibiting antisymmetry (table S1, section a), but can it be biased toward one side by external environmental stimuli? If asymmetry direction is purely random, then antisymmetry merely reflects stochastic development. But if environmental stimuli can bias asymmetry toward one side, then dextral and sinistral phenotypes unequivocally represent environmentally induced states. More studies like those of the late C. K. Govind (*64*) are essential: In lobsters, differential use during a short-lived juvenile stage determines which claw becomes the crusher claw. Indeed, if one claw is not sufficiently stimulated by food or object manipulation, then no crusher claw develops at all. Predictable behavioral asymmetries (handedness) may also induce morphological asymmetries, as they do in *Cancer* crab claws (*65*), human hands (*66*), and upper arm bones of professional tennis players (*67*). The developmental basis of antisymmetry deserves more attention.

Adaptive importance of directional asymmetry. Directional asymmetry has evolved independently in many groups (*6*, *7*), but why is predictable asymmetry favored over random asymmetry? Directionally asymmetric environments seem limited to special cases (e.g., the overwhelming excess of dextral gastropod shells undoubtedly influences the direction of hermit crab asymmetries).

Conspecifics may sometimes generate the most predictable asymmetry in an individual's environment. If so, positive frequency-dependent selection could favor one enantiomorph. For example, in male fiddler crabs, which are typically antisymmetric, crabs of opposite handedness sometimes have longer or more injurious fights (*68*). Dextral males therefore would be favored anywhere dextral males predominated, even if by chance. Then, any heritable variation for direction of asymmetry should amplify the frequency of one enantiomorph, as likely happened during the origin of the dextral subgenus *Gelasimus* (*69*). Similarly, in fish where males possess an asymmetric clasping or intromittent organ, females may also be asymmetrical (*70*). Here too, mutations that biased asymmetry toward one side would be favored anywhere one enantiomorph of the opposite sex was more common, even if only by chance initially. This probably occurred repeatedly in pulmonate land snails, where compatibility of coiling direction affects mating success and likely promotes reproductive isolation (*71*).

Advantages of coordinated development may also favor the fixation of one enantiomorph in which multiple asymmetrical traits interact. For example, in humans, complete *situs inversus* (the concordant reversal of all asymmetrical internal organs) poses few clinical hazards, whereas heterotaxias (discordant asymmetries in different organs) can cause serious problems (*37*). Genes promoting asymmetry in one direction might increase concordance of visceral asymmetries and therefore avoid potentially dangerous heterotaxias.

Finally, ubiquitous structural or functional asymmetries in cytoskeletal molecules (*72*) may favor some asymmetrical patterns of cell growth, movement, or division over others because of greater stability or coordination of development, such as the cytoskeletal elements in the egg cytoplasm that influence direction of spiral cleavage in

snail embryos (*73*). We know far too little about the adaptive importance of directional asymmetry.

Prevalence of genetic assimilation. Is genetic assimilation as common among other traits as among biological asymmetries? Regrettably, because compelling evidence is difficult to obtain, rather few natural examples are known. They include (i) the shell shape in freshwater snails, which responds plastically to turbulence in some populations but not others (*74*); (ii) viviparity in reptiles, in which some optionally retain eggs in the oviduct and others consistently do so (*75*); (iii) sex determination in turtles, in which environmental control is ancestral, but genetic control has evolved six times (*76*); (iv) leaf form in buttercups, in which some populations produce aquatic or aerial leaves in response to growth conditions, but others grow only aerial leaves (*77*); and (v) ant-attracting extra-floral nectar secretion in *Acacia* trees, which occurs only after insect attack in some species but is continuous in derived (obligate ant-hosting) species (*78*). Genetic assimilation may be much more widespread than currently believed (*11*).

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