

Figure 1. Molecular interactions of the BBSome. See text for details.

problems and defects in mucus clearance that are characteristic of primary ciliary dyskinesia, a disorder of motile cilia.

Anything related to signaling? This is one of the most exciting aspects of primary cilium biology and BBSome function. In the ear of *bbs* knockout animals, hair cells frequently fail to align with one another, a characteristic of defective planar cell polarity (PCP). Although cilia are now known to be essential for PCP in vertebrates, no one knows the identity of the relevant signals sensed and transduced by cilia. Do cilia sense a morphogen gradient that instructs polarity within the plane of the epithelium? Or are cilia facilitating planar cell polarization established at cell-cell contacts by relaying a permissive signal? On the obesity front, *bbs* mutant mice are unable to transduce leptin signals in specialized hypothalamic neurons that control feeding behavior. Since the leptin receptor was found to interact with a BBSome subunit, it has been hypothesized that the leptin receptor may get trafficked to cilia by the BBSome. However, to this date, no one has succeeded in visualizing the leptin receptor in cilia of the relevant neuronal cell types. Nonetheless, the discovery that IFT dysfunction also causes unregulated weight gains in mice makes the ciliary hypothesis of leptin signaling extremely appealing.

What remains to be explored?

Nearly everything! What are the membrane proteins that require the BBSome for their trafficking? Does the BBSome function only in trafficking to cilia or is it also involved in IFT or trafficking out of cilia? What is the molecular activity of the BBSome? Does it have any enzymatic activity? What is the function of the BBS proteins that do not belong to the BBSome? In particular, does BBS3/Arl6 function in vesicular trafficking? How and where is the BBSome assembled? Do the type II chaperonin-like BBS proteins BBS6, BBS10 and BBS12 play a role in the folding or assembly of BBSome subunits?

Where can I find out more?

- Fliegauf, M., Benzing, T., and Omran, H. (2007). When cilia go bad: cilia defects and ciliopathies. *Nat. Rev. Mol. Cell. Biol.* 8, 880–893.
- Nachury, M.V., Loktev, A.V., Zhang, Q., Westlake, C.J., Peränen, J., Merdes, A., Slusarski, D.C., Scheller, R.H., Bazan, J.F., et al. (2007). A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* 129, 1201–1213.
- Pazour, G.J., and Bloodgood, R.A. (2008). Targeting proteins to the ciliary membrane. *Curr. Top. Dev. Biol.* 85, 115–149.
- Seo, S., Guo, D.F., Bugge, K., Morgan, D.A., Rahmouni, K., and Sheffield, V.C. (2009). Requirement of Bardet-Biedl syndrome proteins for leptin receptor signaling. *Hum. Mol. Genet.* 18, 1323–1331.

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Primer

Animal asymmetry

A. Richard Palmer

For decades morphological asymmetries have evoked curiosity and wonder (Figure 1). Although largely studied by natural history connoisseurs, many wonderful stories emerged: for instance, lopsided flatfish that lie on one side of their body and have both eyes on the other; the narwhal's spectacular, sinistrally-coiled and left-sided tusk; *Velevella velevella*, the by-the-wind sailor that drifts on the ocean surface and has right- and left-sailing forms; the ability of oppositely coiled snails to mate — sometimes it's easy and sometimes it's not; male theridiid spiders that rip off one palp and eat it, leaving only one for mating; male fiddler crabs with a massive claw (up to 40% of body weight) that is used for signaling and fighting.

Morphological asymmetry is one of those exceedingly rare characteristics of animals (and protists and plants) that has evolved independently many times (Table 1). In a 1932 compilation not since equaled, Wilhelm Ludwig tallied all known examples and kinds of animal asymmetries: large, small, bilateral, helical, morphological and behavioral. But little general insight emerged from this Herculean exercise other than an attempt to standardize terminology, some speculations on common causes, and a nearly 100-page screed on handed behavior in humans and other primates, a subject that, astonishingly, remains poorly understood even today.

A simplified perspective on morphological asymmetry

Despite the great diversity of asymmetrical forms, a focus solely on direction of asymmetry renders broad-scale comparative studies of asymmetry variation tractable. This is because the development and evolution of a simple and well-defined qualitative trait — direction of asymmetry — can be easily compared among organisms with very different body plans. If only direction is considered,

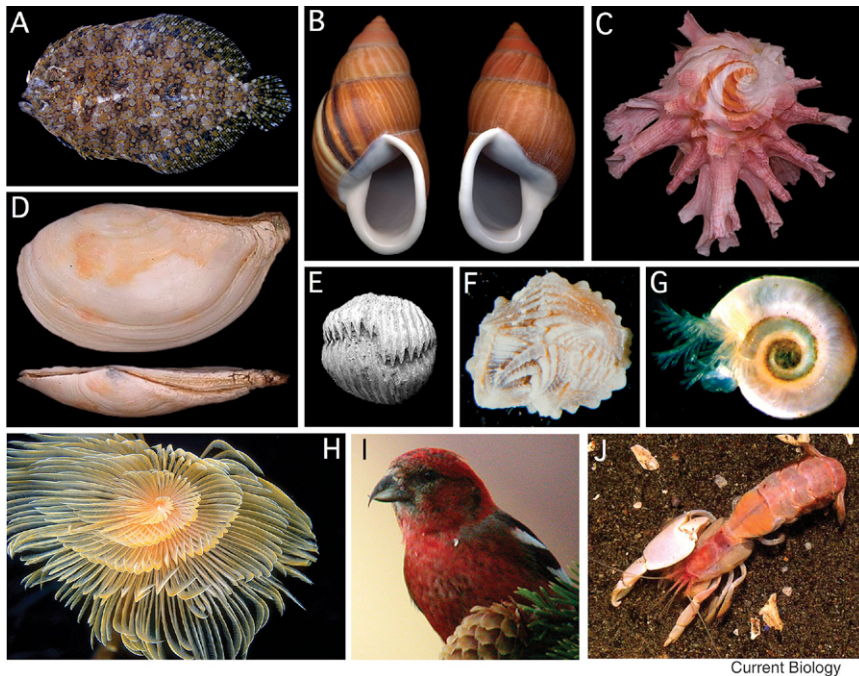


Figure 1. Examples of morphological asymmetries in different animal groups.

(A) *Bothus lunatus*, a flatfish whose eyes lie on the left side of the head (photo by Arthur Anker). (B) *Amphidromus heerianus*, a polymorphic snail having both dextral (left image) and sinistral (right image) forms (photo by Bill Frank). (C) *Chama lazarus*, a bivalve that cements one valve to the substratum, in this case, the left one (photo by George Sangiologlou). (D) *Pandora inaequiv-alvis*, a bivalve that lies horizontally near the sediment-water interface on either the right or left valve (photo with permission from www.femorale.com). (E) *Torquihynchia*, a brachiopod where the dorsal (upper) and ventral (lower) valves are raised on one side (right side of valve in this image) at random (reproduced with permission from Fürsich, F. T. and T. Palmer (1984). Commis-sural asymmetry in brachiopods. *Lethaia* 17, 251–265. With permission from Wiley-Blackwell). (F) *Verruca* sp., a peculiar group of barnacles with only two movable opercular plates, the remaining two (right or left at random) are fused into the rigid ring of lateral wall plates. (G) *Circeis americana*, a coiled tube-building polychaete (Spirorbinae); tubes attach to the substratum along the dorsal or ventral side so the worm's body bends to the right or left (photo by Tara Macdon-ald). (H) *Spirographis*, a feather-duster type polychaete worm with a coiled tentacle fan (photo by Christophe Quintin). (I) *Loxia leucoptera*, a crossbill finch where the upper mandible crosses randomly to the left or right of the lower one (to the left here) (photo by Frode Falkenberg, www.cyberbirding.no). (J) *Neotrypaea californiensis*, a large male thalassinid mud shrimp with a greatly enlarged right claw (photo by Greg Jensen).

three types of conspicuous asymmetry typically occur within species: dextral (all individuals right-sided or dextrally coiled), sinistral (all individuals left-sided or sinistrally coiled) or random (half of the individuals are right/dextral and half are left/sinistral; sometimes called antisymmetry). An even simpler grouping is: fixed asymmetry (all individuals asymmetrical in the same direction, regardless of direction) and random asymmetry. One or more of these kinds of asymmetry occur in the external form of many animal groups (Table 1), as they do in internal organs.

Two questions emerge: of what significance is the observation that direction of asymmetry is fixed in some species and random in others?

What role do genes and environment play in the evolutionary origin of morphological asymmetry (i.e., in the breaking of symmetry during development when morphological asymmetries first appear evolutionarily)?

Fixed asymmetry: inheritance

Fixed asymmetries occur in many groups (Figure 1, Table 1), and may be either right-sided (dextral) or left-sided (sinistral). In most such species, reversed individuals occur occasionally. These variants permit breeding studies to test whether direction of asymmetry is controlled by a few or many genes. The answer is mixed. In all snails studied so far, coiling direction is inherited predominantly as a single-locus,

two-allele polymorphism, a result made somewhat puzzling by the fact that either *dextral* or *sinistral* may be dominant. In flatfish, eye-side inheritance has only been studied in starry flounder, a rare polymorphic species whose eye-side frequencies depart significantly from random. Eye side is clearly heritable, but, curiously, only about 70%. Some odd observations in cultivated flatfish raise eyebrows even further. Despite the rarity of reversed individuals in nature, flatfish in cultivation exhibit up to 20% reversal, suggesting that eye-side determination remains sensitive to environmental effects.

Another peculiar mode of inheritance involves an internal, anatomical asymmetry. Like all vertebrates, mice have an asymmetrical, left-sided heart. Curiously, heart side is random in *iv* mutant mice, regardless of the parents' direction of asymmetry. Therefore, unlike snails, the two alleles are not for *left* and *right*, but for *left* and *random*.

Random asymmetry: inheritance

A more consistent, albeit surprising, pattern of inheritance emerges from studies of random asymmetries (Table 1, Figure 1). Because dextral and sinistral forms are equally common in such species, tests for inheritance are easier to conduct. Remarkably, among 13 animal studies only one suggested that direction of asymmetry was inherited, and doubts remain about that exception. Add to this even more results from plants — in 15 of 16 cases direction is not inherited — and a broad generalization emerges. With only one exception — albeit a highly informative one (see below) — direction of asymmetry is not inherited in cases of random asymmetry.

In cases of random asymmetry, therefore, right-sided and left-sided are conspicuous phenotypic variants that almost always lack a genetic basis. This lack of a heritable basis to direction of asymmetry raises some fascinating questions about how right and left forms develop. Perhaps direction is entirely stochastic, or random influences from the environment induce asymmetry in a particular direction in an individual.

Ontogeny of asymmetry

Two examples of how morphological asymmetries develop, one fixed and one random, show how symmetry-breaking is coupled developmentally to other asymmetries, either in the intracellular environment or in influences from the external environment.

In some gastropods, the orientation of shell coiling may be traced back ontogenetically to the orientation of cleavage planes in early spiral cleavage. In 1895, Henry Crampton first noted that spiral cleavage orientation was reversed in a sinistral gastropod, *Physa heterostropha*, compared to the more typical orientation in the vastly more numerous dextral gastropods — and, indeed, in most other spirally cleaving animals. In a classical study with the polymorphic freshwater snail *Lymnaea peregra*, Gary Freeman and Judith Lundelius confirmed that coiling direction was inherited predominantly as a single-locus two-allele polymorphism, with dextral being dominant. They also showed that embryos from genetically sinistral mothers exhibited a reversed orientation of spiral cleavage compared to embryos from genetically dextral mothers (in snails, the asymmetry phenotype of the offspring reflects the genotype of the mother). Therefore Crampton's original observations of differences among species also apply to shell coiling direction within species. Most remarkable of all, motivated by a hunch that the recessive sinistral allele was a loss-of-function allele, Freeman and Lundelius transplanted egg cytoplasm from fertilized eggs of dextral mothers into those of sinistral mothers and managed to reverse spiral cleavage orientation. Some cytoplasmic (likely cytoskeletal and chiral) component in the egg is clearly responsible for orienting spiral cleavage and, ultimately, body asymmetry and shell coiling. Regrettably, the identity of this factor remains elusive.

American lobsters (Figure 2), much appreciated as a culinary delicacy, are a textbook example of random asymmetry. In large samples, half have the large crusher claw on the left side and half on the right. Simple yet elegant laboratory experiments

Table 1. Selected examples of conspicuous external morphological asymmetries in animals.*

Taxon (example)	Asymmetric trait	Asymmetry type**		
		R	L	R+L
Mammals				
Cetacea (whales, dolphins)	Dorsal skull midline deviation		X	
Monodontidae (narwhal)	Side of elongate tusk in male		X	
Birds				
Carduelinae (crossbill finch)	Side of upper mandible tip			X
Charadriidae (wry-bill plover)	Direction of bill twist	X		
Strigiformes (owls)	Larger/higher ear opening	X	X	
Reptiles				
Serpentes (snakes)	Side of larger hemipene	X		
Fish				
Pleuronectiformes (flatfish)	Eye side	X	X	X
Phallostethidae	Side of male clasper (priapium)	X	X	X
Scale eating species of Cichlidae, Triacanthodidae and Characidae	Side of mouth deflection			X
Cephalochordates (lancelets)	Side of larval mouth		X	
Hemichordates (acorn worms)	Side of proboscis pore (if single)		X	
Echinodermata				
Larvae of all classes (exc. crinoids)	Side of juvenile rudiment		X	
Brachiopods				
Orthida† and Rhynchonelloida†	Dorsalmost side of gape			X
Bryozoa (moss animals)				
Fenestrata† (<i>Archimedes</i>)	Colony coiling direction			X
Gymnolaemata and Stenolaemata	Colony coiling direction			X
Crustaceans				
Cirripedia (verrucomorph barnacles)	Side of lost lateral plates			X
Copepoda (looking glass copepod)	Side of 'black organ' + others			X
Ostracoda (seed shrimp)	Side of larger valve	X	X	
Malacostraca- Decapoda				
Astacidea (clawed lobsters)	Side of larger first claw			X
Caridea (snapping and river shrimp)	Side of larger first claw			X
Thalassinidea (mud shrimp)	Side of larger first claw	X	X	X
Brachyura (true crabs)	Side of larger first claw	X	X	X
Anomura (hermit crabs)	Side of larger first claw	X	X	
Insects				
Orthoptera (katydids, crickets)	Side of wing cover with file	X	X	
Thysanoptera (thrips)	Side of mandibular stylet		X	
Coleoptera (carabid beetles)	Side of notched mandible	X	X	X
Many insect orders	Male genital asymmetry	X	X	X
Chelicerates				
Arachnida (theridiid, pholcid spiders)	Side of palp in male			X
Acari (feather mites)	Side of elongated legs			X
Polychaete Annelids				
Spirorbinae (coiled tube worms)	Coiling direction of tube	X	X	X
Serpulidae (calcerous tube worms)	Side of opercular plug			X
Sabellidae (feather duster worms)	Coiling direction of fan			X
Cephalopods				
Coleoidea (squids, octopus)	Side of hectocotylus in male		X	X
Ammonoidea† and Nautiloidea†	Shell coiling direction	X	X	
Bivalves				
Anomiacea, Pectinacea	Attached/smaller side		X	
Ostreacea, Chamacea, Hippuritacea	Attached/larger side	X	X	X
Gastropods				
Prosobranchia (marine snails)	Coiling direction of shell	X	X	X
Pulmonata (land snails)	Coiling direction of shell	X	X	X
Hydrozoan Cnidarians				
Hydroida (by-the-wind-sailor)	Direction of sail twist			X
Siphonophora (Port. man-o-war)	Direction of sail twist			X

*For each taxon, an X indicates at least one example is known; this does not mean this form of asymmetry is representative of all species in that taxon. **R, right, dextral or clockwise; L, left, sinistral or counterclockwise; R+L, random asymmetry (both R and L forms found commonly within species, typically in equal frequencies). †Extinct species only.

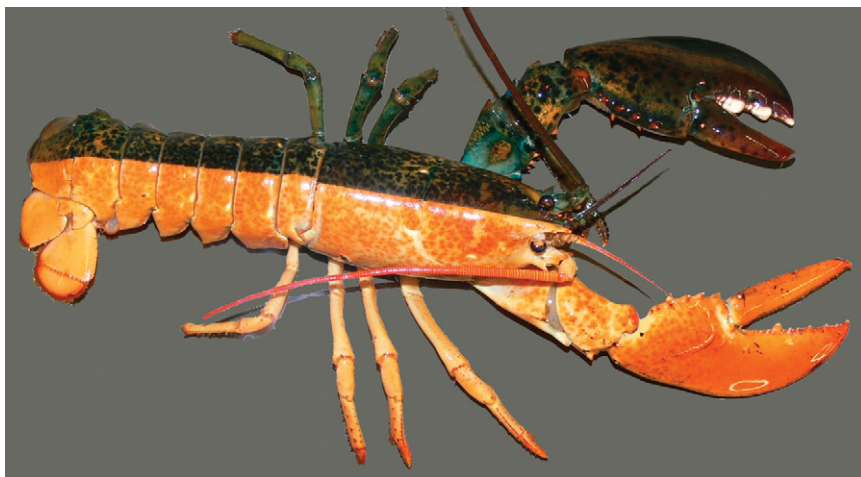


Figure 2. An anomalous two-toned American lobster. This *Homarus americanus* possesses asymmetrical claws (left side: crusher type, right side: cutter type), and a stunningly crisp midplane (background digitally removed).

by the late C.K. Govind showed how differential claw use by juveniles, which initially have symmetrical claws, induces one to transform into a crusher claw. Young juveniles reared through five molts with hard objects to manipulate or with a second small lobster for interaction develop the crusher claw at random on the right or left. Autotomy or denervation of the right claw induces a crusher claw on the left. Preferential exercise of the left claw induces it to transform into a crusher claw. Most amazing of all, without sufficient stimulation during the critical developmental window for symmetry breaking, neither claw becomes a crusher claw. Clearly, the developmental program that yields a crusher claw still requires an appropriate environmental trigger to initiate it.

This example raises an interesting question: does handed behavior induce or orient morphological asymmetry during ontogeny? If lateralized behaviors, such as preferred use of the right or left limb for feeding, are learned, and if differential use induces differential development of one side in the same way it enhances development of many structures, such learned behaviors might greatly facilitate both the ontogeny and evolution of morphological asymmetry.

Evolution of asymmetry

Phylogenetic studies of asymmetry variation offer surprising insights into

how developmental mechanisms evolve. They address a question that might, at first glance, seem innocuous: Did animals with fixed morphological asymmetries evolve directly from symmetrical ancestors or from ancestors that exhibited random asymmetry? But this question actually represents a fundamental one in evolutionary biology: which comes first evolutionarily, mutations that yield novel phenotypes, or novel phenotypes, followed later by mutations that facilitate their development? In other words, from the perspective of left-right asymmetry, are mutations for rightness or leftness what generates new right- and left-sided phenotypes, or do new right- and left-sided phenotypes arise first, followed by mutations that stabilize development of rightness and leftness?

A simple comparative test of these alternative modes of evolution is possible by way of a single assumption. In species that exhibit random asymmetry, we assume that direction of asymmetry is not inherited, at least in the absence of direct evidence for inheritance. This assumption seems safe because it has been verified in 28 of the 29 cases examined (see above). Moreover, the sole exception — style bending in enantiostylous flowers of some monocot plants — actually confirms that genetic control of bending direction appeared evolutionarily after the bent-style

phenotype already existed, a pattern seen in many taxa that include both random and fixed asymmetries.

If direction of asymmetry is inherited in cases of fixed asymmetry but not inherited in cases of random asymmetry, two evolutionary scenarios are possible. If a species with fixed asymmetry evolved from a randomly asymmetrical ancestor then mutations that induce right-sidedness or left-sidedness most likely arose evolutionarily after the conspicuous morphological phenotypes right-sided (dextral) and left-sided (sinistral) already existed as a polymorphism. Alternatively, if a species with fixed asymmetry evolved directly from a symmetrical ancestor, then mutations that induce *right-sidedness* or *left-sidedness* likely initiated the morphological asymmetry.

Take flatfish (Pleuronectiformes), for instance: as adults, they lie horizontally on one side of their body, with both eyes facing upwards on the other. Yet they begin life like any other planktonic fish larvae, swimming upright with two eyes symmetrically placed on opposite sides of the head. Then, as they approach the time to settle they pass through an extraordinary transformation. One eye actually migrates across the midline of the skull to lie wholly on the other side of the head. Only then, with both eyes firmly ensconced on one side, do they settle into life on the bottom.

The evolutionary history of flatfishes (Figure 3) is at least as remarkable as their ontogeny. Two of the three most ancient extant lineages exhibit random eye-side asymmetry. Add to this the recent report of random asymmetry in early fossil flatfishes, and it seems very likely that random eye-side asymmetry was the ancestral state. From random-eyed ancestors, both right-eyed and left-eyed species arose independently at least twice, with occasional evolutionary reversals of direction or reversions to polymorphism in both crown clades (I and II). Eye-side direction is clearly an evolutionarily labile trait.

One startling conclusion emerges from this evolutionary history: genes directing larval flatfish to become right-eyed or left-eyed likely arose evolutionarily after conspicuously right-eyed and

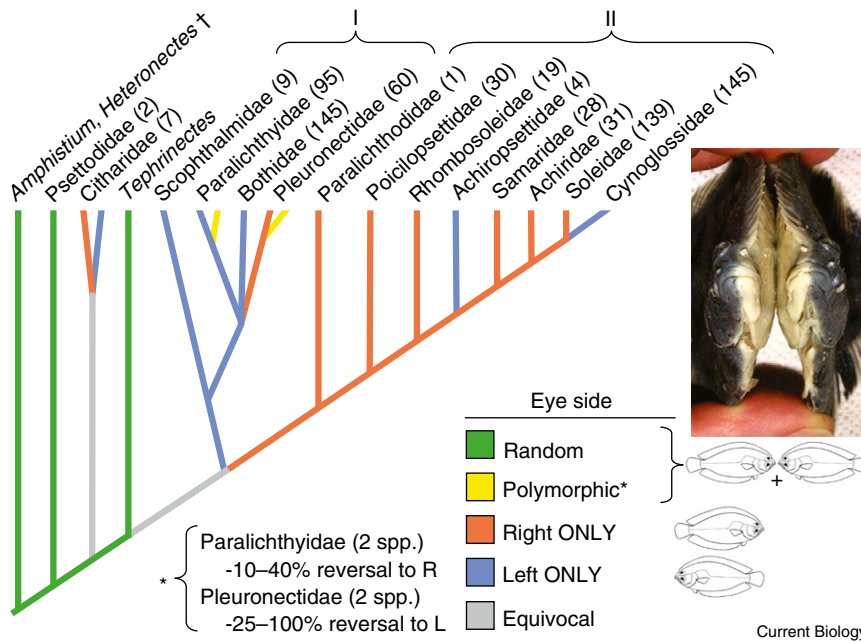


Figure 3. Evolutionary relations among living and fossil flatfish.

Numbers of living species are given in parentheses. Two basal clades of living flatfish (Psettoidea, *Tephriinectes*), and both genera of extinct fossil flatfish (†), all exhibit random asymmetry (green), so random asymmetry was the ancestral state in the Pleuronectiformes. From this ancestral state, right-eyed (red) and left-eyed (blue) descents evolved independently in both the Citharidae and in the crown flatfish groups (I and II). Therefore, genes for direction of asymmetry (red and blue lineages, where eye-side is fixed) arose evolutionarily *after* right- and left-eyed flatfish already existed but where eye-side was random and not inherited (green lineages). Eye-side reversals have also occurred evolutionarily at least three times (Pleuronectidae, Achirosettidae, Cynoglossidae). ‘Polymorphic’ species differ from randomly asymmetrical species because their eye-side frequencies depart significantly from random. Inset: anterior views of right-eyed (left) and left-eyed (right) individuals of the polymorphic flatfish *Platichthys stellatus*; the anatomical dorsal side is up, but in life both individuals would lie horizontally with their light-colored side on the substratum (photo by Carolyn Bergstrom).

left-eyed flatfish already existed. Random eye side in the earliest flatfish very strongly suggests that eye-side was determined either purely stochastically or by randomly lateralized environmental cues. Only later did genes arise that biased eye-migration predictably towards a particular side of the head. In other words, in flatfishes — a wholly novel form of fish if there ever was one — eye-side asymmetry appears to exhibit a phenotype-precedes-genotype mode of evolution during the early radiation of the group.

When this logic is applied to many clades of animals, an even more surprising result emerges: between one-third and one-half of the cases of fixed asymmetry (where genes play a role orienting the asymmetry in a particular direction) arose evolutionarily from ancestors that exhibited random asymmetry (where direction of asymmetry is

typically not inherited). So, fixed asymmetries evolved almost as often via a phenotype-precedes-genotype mode of evolution as via the more conventional genotype-precedes-phenotype mode.

Despite their evident success (over 700 living species), and despite our progress in understanding their evolutionary history (Figure 3), one big question remains: what possible advantage is there to having eyes on the right side of the head versus the left, or vice versa? Eye side became genetically fixed presumably because individuals carrying genes for right-eyedness in some lineages and left-eyedness in other lineages somehow had higher fitness. But any invoked advantages must account for how eye-side became fixed to one side from some random-eyed ancestor, and how right-eyed descendants evolved from left-eyed ancestors, and vice versa.

Of course, these puzzles apply to all cases where fixed asymmetries evolved from randomly asymmetrical ancestors, or where direction of asymmetry changes evolutionarily. But that’s one reason the study of right-left asymmetry remains so fascinating.

Emerging generalities

The flatfish example illustrates nicely how morphological asymmetries offer a rich buffet of puzzles about development, functional morphology, ecology and evolution. By studying the simple characteristic — *direction of asymmetry* — solutions to these puzzles can be compared among groups with highly divergent body plans, including protists, plants and animals. Broad generalities about the interplay between development and evolution, and between genes and environment, seem possible — if only we take the time to look.

Acknowledgments

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Further reading

- Asami, T., Cowie, R.H., and Ohbayashi, K. (1998). Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. *Am. Nat.* 152, 225–236.
- Freeman, G., and Lundelius, J.W. (1982). The developmental genetics of dextrality and sinistrality in the gastropod *Lymnaea peregra*. *Roux’s Arch. Dev. Biol.* 191, 69–83.
- Friedman, M. (2008). The evolutionary origin of flatfish asymmetry. *Nature* 454, 209–212.
- Govind, C.K. (1989). Asymmetry in lobster claws. *Am. Sci.* 77, 468–474.
- Huber, B.A., Sinclair, B.J., and Schmitt, M. (2007). The evolution of asymmetric genitalia in spiders and insects. *Biol. Rev.* 82, 647–698.
- Ludwig, W. (1932). *Das Rechts-Links Problem im Tierreich und beim Menschen* (Berlin: Springer).
- Neville, A.C. (1976). *Animal Asymmetry* (London: Edward Arnold).
- Palmer, A.R. (2004). Symmetry breaking and the evolution of development. *Science* 306, 828–833.
- Palmer, A.R. (2005). Antisymmetry. In *Variation*, B. Hallgrímsson and B.K. Hall, eds. (New York: Elsevier), pp. 359–397.
- Palmer, A.R. (2006). Caught right-handed. *Nature* 444, 689–691.

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