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Reviews

Reflections on the ambivalent helix

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Summary. The helix is nature's favourite shape. Because of its elementary geometry and distinctive appearance it is also the clearest instance of an enantiomorphic object – a helix and its mirror image are identical in all respects except their screw sense. This is a distinction that can be ignored from the points of view of pure geometry and pure group theory[18] but any helical structure is actually available as either or both hands.

Whether in nature helices do occur as just one hand, or both, is one of the best – perhaps the best – puzzles of the science of form. In this short review I look at a few examples of naturally occurring helices, some where only one hand is found, some where both are commonly found, and perhaps the most interesting examples in biological terms – those where both are found but one hand is very much rarer than the other. I review what mechanisms – physico-chemical, genetic, evolutionary – underlie the different manifestations of left- and right-handedness.

Key words. Helix; handedness; enantiomer.

> *It is no accident that (Alice) Through the Looking Glass is filled with references to mirror reversals and asymmetric objects. The helix itself is mentioned several times....*
> Martin Gardner (The Wasp in a Wig, 1977)[33]

A helix "goes the other way in a mirror" – to use Alice's own words. In theory at least then, any helix is one of a pair of identical 'twins', identical that is except that one is left-handed and the other right – they are enantiomers, mutual mirror images that cannot be superposed. Whether a helix possesses an actual twin (or even a counterpart) is I believe one of the best questions of the science of form – possibly its deepest (fig. 1.).

The potential for having a mirror-reversed twin is not, it should hastily be added, peculiar to helices with their screw symmetry. It is characteristic of any object that possesses no inverse symmetry elements. Many molecules exist as enantiomers. A few are helical. Most are not. The most familiar instance of enantiomorphism is that of a pair of hands, which is why 'handedness' (see appendix 2) is attributed to mutual non-superposable mirror images. However the helix is geometrically elementary and readily recognised. It should therefore, reveal most clearly the principles underlying the existence (or not) of mutual mirror images. Conklin[15], for instance, said in his paper *Causes of Inverse Symmetry* that "inversion of symmetry [i.e. production of a mirror image] in animals, with its profound implications for embryology, is clearly seen in gastropods [which are roughly helical] though doubtless taking place in other animals where it is obscured".

The helix: nature's favourite shape

The helix turns out to be nature's favourite shape – its agreeable economy making it the preferred solution for innumerable problems of growth, form and function in living things. Because it is so common, found at every anatomical level across about 9 orders of magnitude (table 1), it is possible to disentangle to some extent the mechanical or structural design principles behind the helix from the large number of ways the design can be realised. One reason for the popularity of the helix can be found in Needham's[57] rather apt description of biology as "largely the study of fibres". Add to this the idea articulated by Crane[19] that "any structure which is straight or rodlike [a category that includes fibres when the length greatly exceeds the diameter] is probably a structure having a repetition along a screw axis", i.e. a helix, and the crucial and central role played by the helix in biology is seen clearly and emphatically. Much more recently, Wainright et al.[72] have pointed out that the hollow cylinder provides the most common design for body walls on every scale. The cylinder lends itself to a helical mode of construction whether using discrete subunits – as in the cylindrical viruses – or helical winding as in animal bodies, and plant cell walls (fig. 2).

"Twas brillig, and the slithy toves . . . ."

Figure 1. Twas brillig and the slithy toves.... Two toves can be seen in this picture which Sir John Tenniel painted for *Through the Looking Glass*. They have helical noses and tails. Both toves appear to be left-handed. No other illustrations being extant it is not known whether there exist right-handed as well as left-handed toves (Courtesy MacMillan Press).

What is a helix? Some geometry

It will probably be helpful now if I define my terms, and also relate the idea of the helix to that of the spiral. The two words are often used as though they are interchangeable although a spiral staircase is really a helical one and in botany, spiral phyllotaxis is usually the arrangement of leaves on helical paths. For the purpose of this review I will take it that 'spiral' refers to coiling in a single plane and 'helix' to a coiled form which advances around a central axis.

In strict geometrical terms "a helix is a curve drawn on a cylinder so as to cut the generators at a constant angle. In other words it is produced by drawing a straight line on a sheet of paper and then wrapping the paper on a cylinder"[18].

Often the cylinder is erected on a circle and for many people 'helix' is synonymous with a curve produced in this particular way. However this helix ought, strictly speaking, to be called a circular helix. In fact much more interesting helices are produced when the cylinder is erected not on a circle or some other simple closed curve but on a spiral. A well-known helix in biology results when the spiral is the logarithmic or equi-angular spiral. Since this is an important curve it would be as well to be clear about its nature.

In polar coordinates (r, ϑ) the logarithmic spiral is prescribed by $r = a\mu^{\vartheta}$ where a and μ are both constant. The

Table 1. A striking feature of helical structures is that they arise at every anatomical level from the molecular, the single α-helix say, at the limits of resolution of the electron microscope, up to the visible – and the very large. Thinking of the helix as the obvious way to build a cylindrical stick or tube the table below gives examples of the diameter of such cylinders across about nine orders of magnitude.
Notice the range of diameters within each class of structure: for molecules the range is covered by a factor of about two; for molecular tubes, roughly an order of magnitude; for cells it is two orders or so; for whole animals the range is greater than three orders of magnitude, perhaps even four.

Type of structure		Example	Diameter of helix, nm
Fibre	Molecules	α-helix	1.0
		Collagen triple helix	1.5
		DNA double helix	2.0–2.5
Hollow tubes; assemblies of (usually) globular protein molecules	Viruses/organelles	Bacterial filamentous virus	6
		Potato virus X	13
		Tobacco mosaic virus	20
		Microtubules	25
		Papilloma/polyma tubes	30–45/55
		Turnip yellow virus polyhead	80
Hollow tubes made by winding fibres	Cells	Aquaspirillum (a bacterium)	$5 \cdot 10^2$–$5 \cdot 10^3$
		Cotton fibre	$3 \cdot 10^4$–10^5
	Animals	Worm cuticle, e.g. of *Wolstorffii parachordes*	$7.5 \cdot 10^5$
		Eel skin	10^7–10^8
		Squid mantle	$\sim 10^8$
		Whale skin	$\sim 10^9$

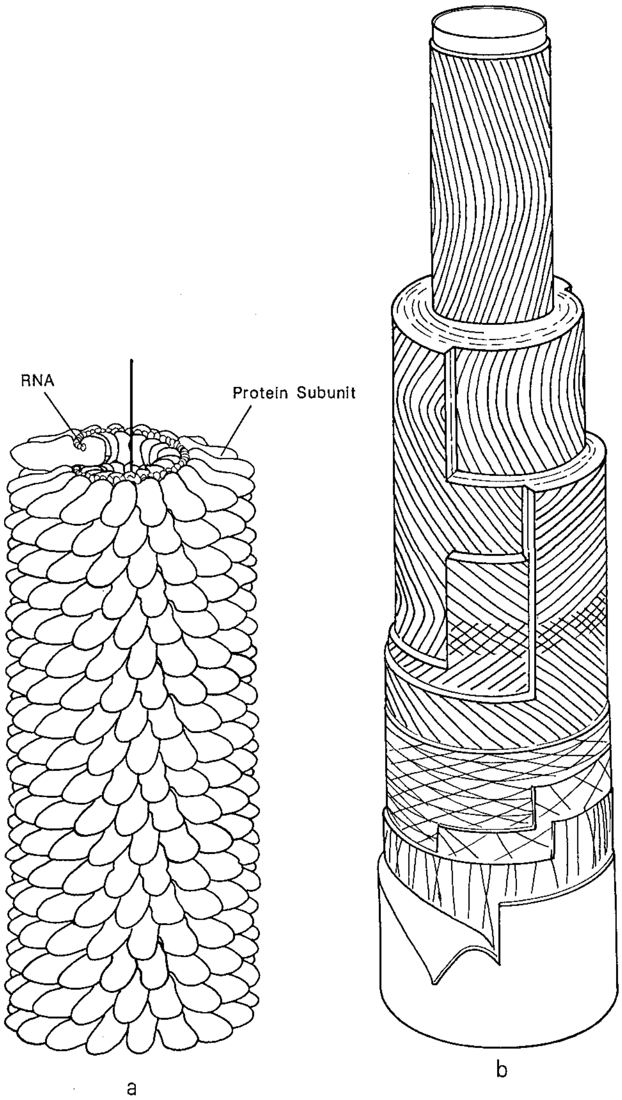

Figure 2. Two kinds of helical structure. *a* Tobacco Mosaic Virus (TMV): A hollow tube constructed of globular protein molecules arranged on a continuous helical path – the genetic or ontogenic spiral. In TMV it is right-handed. The viral RNA follows this helical path. About 16½ proteins constitute the pitch[8]. *b* Cotton fibre cell: a complex multi-layered cylindrical structure made by winding polysaccharide fibres. The fibres follow both right- and left-handed paths. They often abruptly change hand[73].

angle ϕ between the position vector at any point and the tangent to the curve at that point is

$$\cot^{-1}\left(\frac{1}{r}\frac{dr}{d\vartheta}\right)$$

$$\frac{dr}{d\vartheta} = r \ln \mu$$

and it follows that $\tan \phi$ is a constant, $(\ln \mu)^{-1}$ hence the spiral's being called equi-angular.

The helix drawn on the surface of a cylinder erected on the equi-angular spiral is usually called a conchospiral since typically it is the curve upon which the shells of gastropods (and some other animals) are closely based. (Latin for shell is concha). Raup[61] has analysed the conchospiral, most thoroughly and with the most insight. The circular helix is defined by the pair of relationships in cylindrical coordinates,

$$r = a$$

$$Z = c\vartheta$$

where **a** is the radius of the helix and its pitch, P, the distance along the axis after which it repeats is $2\pi\mathbf{c}$. Whereas **a** is constrained to be a positive number, **c** may be either positive or negative and depending on which it is, the helix is right-handed – the curve rises to the right – or left-handed. Exactly the same is true for the conchospiral

$$r = a\mu^{\vartheta}$$

$$Z = c\mu^{\vartheta}$$

or for any other helix for that matter.

An alternative way of looking at the concho-spiral is as a cone of semivertical angle $\psi = \tan^{-1}(a/c)$ intersecting the equi-angular cylinder. Using this idea, changing the sign of the parameter, **c**, turns the cone upside down, again reversing the helix hand. In many gastropod shells the cone shape can be seen very clearly. In others, because they are rather ornate, it is obscured. The conchospiral does not possess a constant pitch but rather, successive turns of the arc cut off segments of the generators of the cone that increase in a geometric progression.

Horns: mirror image pairs of helices

The helical shape of some structures seems clearly related to their possessor's bilateral symmetry. The horns – similar to conchospirals – of sheep, goats and antelopes appear in matched pairs, a left-handed helix on one side of the head, a right-handed helix on the other. In some species the right-handed helix is on the animal's right (described as homonymous) and in others the left-handed helix is on the right (heteronymous). A narwal's single horn usually seems to be left-handed. Cook[16] claimed that the striking thing is that the horns of wild animals are heteronymous whereas those of domesticated species and varieties are homonymous. To explain this he argued that only animals with rapidly spiralling horns would have been domesticated originally – (long, relatively straight horns would be dangerous) – and pointed out that such horns would have to grow forward or they would tend to grow into the animals backs which would presumably be lethal. Thus selection for curling horns would also automatically select for homonymous ones.

Biological macromolecules: helices of one hand only?

The emergence of the helix as the structural paradigm of molecular biology can be traced to Linus Pauling – "rolling paper scrolls on a sick bed in Oxford in 1948 before the helix was built as a model structure by Branson and Corey in Pasadena in 1950...."[38]. Before then the helix was not taken very seriously. Afterwards it became the most common and most important structure for those interested in big biological molecules and recognised as "the classic element of protein structure"[62]. In 1953 biology's most famous helix appeared in print for the first time with the publication of Watson and Crick's[74] paper on the structure of DNA.

Questions that arose immediately and have continued to be asked up to the present day are what hand these molecular helices would adopt – and why. Early structural work on fibrous proteins and nucleic acids relied on fibre diffraction – which could not by itself readily distinguish helix hand. Electron microscope studies of helical viruses, like Tobacco Mosaic Virus (TMV), and helical organelles, like microtubules and bacterial flagella[9] suffered from a similar deficiency although for a rather different reason. The electron microscope could 'see' the back and front of the helix at the same time but could not distinguish which was which. Finch[27] solved this problem for TMV in 1972 for instance.

Although the helix was recognised to be crucial in both proteins and nucleic acids at about the same time, research on the detailed structures of the two molecules and hence the real appreciation of molecular helices, including their sense of twist, had rather different histories. To begin with it was felt that α-helix might appear both right- and left-handed, it apparently having no preferred sense of twist. Linus Pauling's book *The Nature of the Chemical Bond*[60] showed both left- and right-handed α-helices (fig. 3). Cohen[13] discussing optical rotation by globular proteins suggested that optically opposing – i.e. left- and right-handed – chain configurations might produce 'cancelling' effects to explain what was felt to be their low-rotating power. However Elliott and Malcolm[25] later concluded on the basis of optical rotation that α-helix was probably right-handed.

Once high resolution information started to become available from the early 1960's for a large number of globular proteins two things became clear. 1) these molecules contained a lot of helix, mostly α-helix; but other helices were also found, the π-helix, the 3_{10}–and the α_{II}-helix, all realisations of a formula suggested by Bragg and his co-workers in 1950[6]. This gives the number $R = 3n + 4$ of atoms in a hydrogen bonded 'ring' where n takes successively the values 1,2,3,4.... Some globular proteins have 60% or even 70% of their amino acids arranged in helical secondary structure[43]. 2) the helix is indeed always right-handed – with so far the single exception of a solitary turn of left-handed helix in the enzyme thermolysin. Indeed, all structural features of proteins where there is a choice of hand tend to favour one hand over the other[62].

Another large family of helical proteins are the collagens, the chief proteins of animal skeletal and connective tissues – although many collagens are now known which play no very obvious structural role. Collagens are triple helices based on repeating triplets of amino acids, glycine $-X-Y$. X is frequently proline and Y hydroxyproline, rarely found outside the collagens. Each amino acid chain – which in the structural collagens of the vertebrates contains more than a thousand amino acid residues is twisted into a left-handed helix with three amino acid residues in its pitch. Three of these helices wind around one another 'plectonemically' to give a structure for which an exact analogy is rope – also a right-handed, three-stranded, tension-bearing fibre.

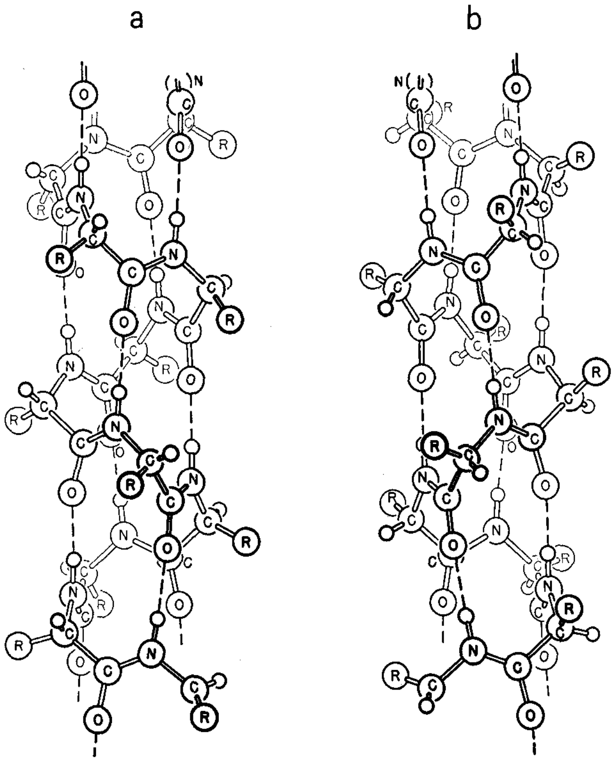

Figure 3. Hypothetical left-, and actual right-handed α-helices. These are not strict mutual mirror images since both are constructed of L-amino acids. True enantiomorphism would require the D form in one helix, the L form in the other. From *The Nature of the Chemical Bond*, by Linus Pauling[60], Oxford University Press, 2nd edn 1960.

(To be precise, 'hawser laid' or 'Z laid' rope is three-stranded and right-handed. Twine is two-stranded. Occasionally rope is four-stranded and also occasionally left-handed or 'S laid'. 'Cable laid' rope is three-stranded and left-handed – formed from three identical strands of right-handed, three-stranded rope laid into one another with the sense of twist reversed.)

The story for the nucleic acids is rather different. Crick and Watson[21] found by trial (and error presumably) that their double helical model for DNA could be built only in a right-handed sense. Steric effects involving van de Waals contacts between the nucleotides apparently ruled out the possibility that the helix was, or indeed could be, left-handed. However it became clear later that in detail their model did not correspond particularly well to any actual observed conformation of DNA – which fell into two families, A and B, differing fundamentally both in the stacking of the base pairs and the nature of the double helical backbone. Fuller et al.[32] reporting part of a thorough and detailed analysis of DNA structure pointed out that the Crick and Watson model resembled the B form rather than the A but that although left-handed helices were very unlikely for the A form it was not in fact easy to rule out the possibility of the B form being left-handed. In the end they fell back on the argument that since A and B forms could be reversibly changed into one another it was very unlikely that such a transition could involve reversing screw sense and concluded that B as well as A must be right-handed. As time went on the picture of helical structures adopted by polynucleotides became more complicated. Considerable conformational freedom is enjoyed by individual nucleotides and this leads to a great range of structural polymorphisms. 'Puckering' of the furanose ring is now seen to account for the differences between the A and B forms for DNA.

Other DNA's were also discovered; some quite early in the history of DNA structures, others later. C and D forms were both identified but turned out to be best thought of as members of the B family and right-handed. The possibility of left-handed helices did not disappear however. A proposal[55] that Poly d (IC) was left-handed – the first concrete proposal for a left-handed DNA – was discredited by Arnott et al.[1]. S and Z DNA's were 'discovered' in the late 1970's in which the structural unit was not a single nucleotide but a pair, one puckered in the form found in A DNA and the other as in B DNA, hence the designation also used of A + B DNA. The important thing about them for the purpose of this review is that S and Z polynucleotides are indeed left-handed helices. These are however very different types of structure from those of the A and B families, they are in no sense left-handed versions of otherwise right-handed helices. A useful article discussing the three families is the one by Dickerson et al.[23] (fig. 4).

Partly because of the availability of improving computing power, partly because of the discovery of left-handed polynucleotide helices, the late 1970's and early 1980's saw an upsurge in theoretical activity aimed at showing on energetic grounds that generally speaking polynucleotide helices could be expected to appear in left- as well as right-handed forms. Useful references can be found in Saenger[65]. It has to be said that whatever might be expected, the A and B families without exception turn out to be right-handed and the A + B left-handed.

Thus we have two major families of protein helices: that dominated by the α-helix; and the collagens; and three major nucleic acid helices: A, B, and A + B. All the members of each family (irrespective of the details – for

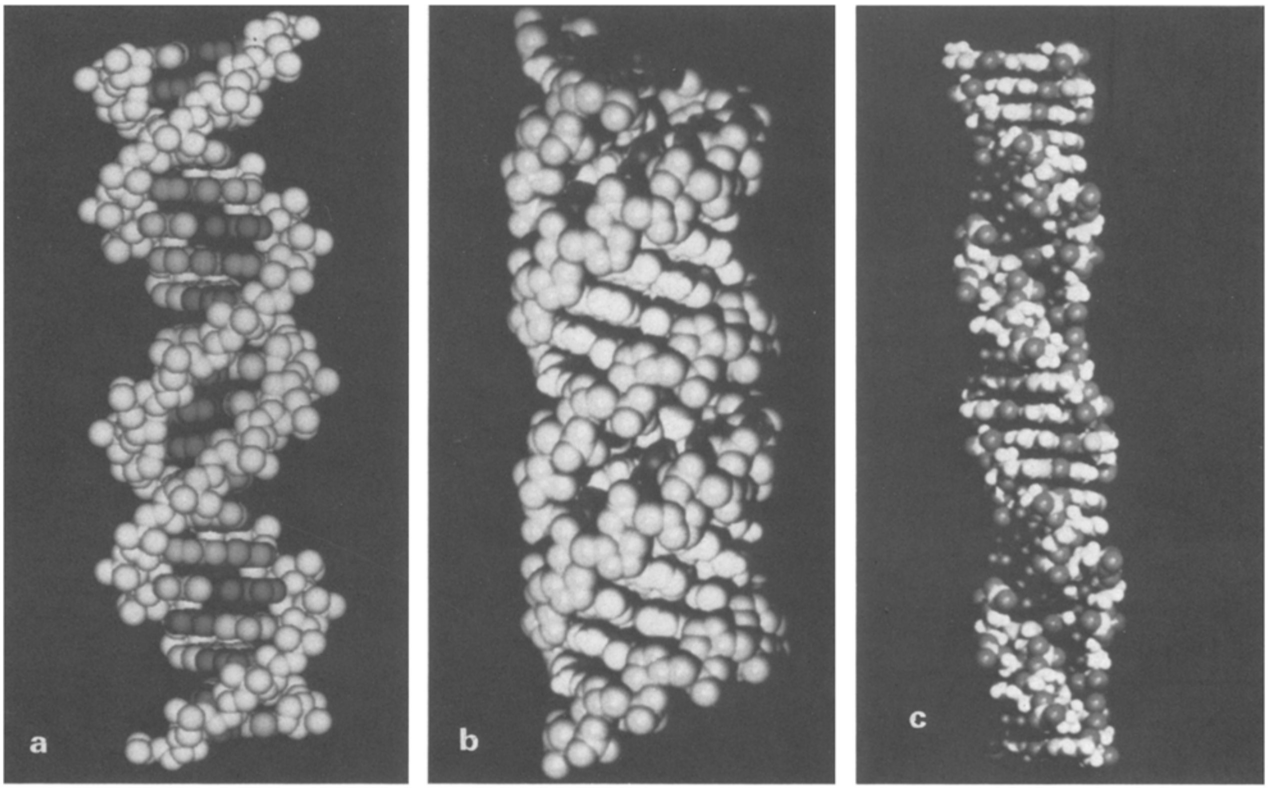

Figure 4. (*a*) A, (*b*) B and (*c*) Z DNA's. A and B are right-handed double helices (duplexes); Z, left-handed. (Computer graphics by kind permission of Dr Rod Hubbard, York University, UK).

proteins, of amino acid sequence and for DNA, nucleotide sequence) possess the same helical hand. For the α-helix and its close relations and for the A and B families of DNA the building block is a single monomer, amino acid or nucleotide. For A + B DNA it is a pair of nucleotides C-G and for collagen a triplet of amino acids ~ Gly Pro Y.

Physical origins of molecular helices

It would be satisfying to be able to give some way of accounting for this great uniformity of behaviour and I want to suggest as a means of doing so the architectural analogy of the spiral – i.e. helical – staircase. Two drawings are reproduced (fig. 5). I found them in Cook's *The Curves of Life*[16]. He in turn had borrowed them from the works of Viollet-le-Duc, the great 19th century French restorer of mediaeval architecture.

Both these staircases are built up by stacking identical structural units which form the successive steps together with a section of the central newel post. In the second, the units are more elaborate than in the first and include a section of handrail as well. The important thing to notice is that in the first staircase the step has a mirror plane parallel to its top and bottom surfaces – its only symmetry element. The 'step' in the second staircase has no symmetry elements at all. Both staircases are shown as right-handed. But by the simple expedient of turning each step over before adding the next the first staircase could be converted to a left-handed one. This is not possible for the second staircase where the shape of the step fixes the hand of the helix quite unambiguously. A staircase of opposing hand could be constructed only from mirror image steps.

The force of this analogy is this. Amino acids – with the exception of glycine – and nucleotides are chiral, i.e. enantiomorphic, molecules. They exist potentially as mirror image pairs but only one hand of amino acid, the L form, and only one hand of nucleotide, also the L form, are found in proteins and DNA. Right-handed (D) amino acids are found in the peptidoglycans that provide bacterial outer coats, and in some antibiotics. In the eukaryotes a single instance of the D amino acid D-alanine occurs in the neuropeptide, dermorphin, found in the skin of some amphibians.

Of the 500 or so naturally occurring amino acids only about 8 are D-forms[71]. The 20 which make up proteins are all L-forms, except glycine which is achiral. It is worth noting that it was as late as 1951 that Bijvoet et al.[3] suggested that anomalous X-ray scattering would discriminate between L and D forms, and allow the hand to be determined absolutely. They showed that the convention guessed by Fischer half a century before for sugar molecules was the correct one, a point made by Crick and Watson (1954)[21] in support of their choice of helical sense of DNA as right-handed.

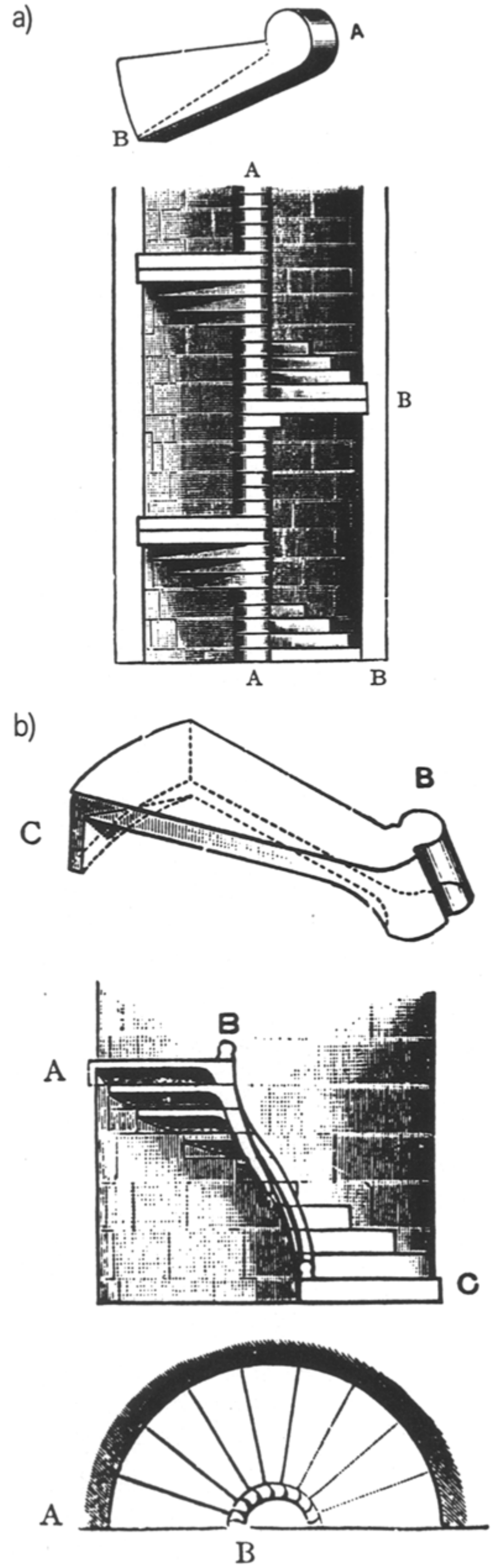

Figure 5. Construction of spiral – i.e. helical – staircases; after Viollet-le-Duc. *a* Steps contain a mirror plane parallel to their upper and lower surfaces. Left- and right-handed staircases can be built from this unit. Equally the steps can be assembled randomly to produce no staircase at all. *b* Steps possess no symmetry elements, are enantiomorphic therefore, and can be assembled to produce only a right-handed staircase[16].

Only L-amino acids occur in proteins and as a result nature favours the right-handed α-helix. Chains of D-amino acids presumably form left-handed α-helices[24]. But what hand of α-helix would an achiral amino acid produce – left or right? Of the 20 or so amino acids found in proteins only the smallest – glycine – is achiral. And it does not form α-helices. Polyglycine crystallizes but does not form independent helical structures in solution.

So not only does the particular hand of the set of individual amino acids dictate the hand of the helix they give rise to, but more profoundly, a single hand appears to be necessary for helices to form at all. No helical protein molecules would mean no collagen – and therefore no skeleton and no muscle – and hence no movement. In fact no helices would mean no higher life forms at all. Thus it is not unreasonable to suppose that the existence of life has depended on evolution being able to choose and then use just one of the two available hands of amino acids and nucleotides. Autocatalysis has been suggested as the way an early imbalance could have been amplified in favour of one hand of amino acid or nucleotide. Joyce et al.[42] for instance have recently studied this possibility in the formation of oligonucleotides.

But what created the original imbalance that marginally favoured left-handed amino acids over right-handed ones. Mason[49] argues that it originates in weak nuclear forces, the result of the non-conservation of mirror image symmetry in fundamental particles and their interactions. He has also argued that this same mechanism might underlie an early observation that left-handed and right-handed quartz crystals do not occur with equal frequency – and that the nucleation of the crystals is biased away from mere chance. However an analysis[31] of the relative frequencies with which both large quartz crystals, and microscopic quartz fibres – which are twisted about a screw axis – supports the idea that nucleation is indeed decided at random.

That the hand of the amino acids determines that of the α-helix suggests that perhaps in turn the α-helical hand determines that of helical assemblies of α-helical molecules. Both myosin and tropomyosin, proteins from which the thick filaments of striated muscle are created are α-helical as is the capsid of the filamentous bacterial virus. Crick[20] made the first suggestion of this sort pointing out that two α-helices would tend to 'cross' at about 20° creating a two-stranded twine-like left-handed helical coiled coil. This double helix was later found to be very common and became firmly entrenched in the minds of molecular biologists – see for example Cohen and Parry[14]. Crossing in relation to packing within globular proteins was analysed by Chothia et al.[10] among others who showed that helices also tended to cross at ~ 52°, a finding which underlies Michel's[53] observation that the folded α-helical molecules of rhodopsin crystallize into helical stacks with 7 molecules in the helical pitch ($7 \times 52 \simeq 360$). The idea that right-handed α-helix forms left-handed helical aggregates – tactoids – and left-handed α-helix, right-handed aggregates was tested by Tachibana and Kambara[68]. They synthesized poly-γ-benzyl D – and L – glutamate, PBDG and PBLG respectively and concluded that PBDG produced aggregates with a right-handed twist and PBLG its mirror image. This was consistent with the D-amino acid producing left-handed α-helix which in turn gave a right-handed aggregate – and vice versa. However, less artificial circumstances give rise to structures less neatly accounted for.

Marvin and his co workers[48] attempted to build the structure of the bacterial filamentous virus Pf1 whose capsid (coat) is constructed from a large number of α-helical structural subunits (capsomeres). They were unable to determine the hand of the viral helix experimentally but fixed it as left-handed on the basis of the presumed interactions between the component α-helices. Later, using improved methods of structure determination they were astonished to discover the virus was right-handed not left-handed and that neighbouring α-helices 'crossed in an unusual negative sense'[7]. A second counter example may be provided by the collagenous ceratotrichia – fin rays – of dog fish and sharks which always dry into right-handed helical structures[51]. (The collagen molecule is also a right-handed helix.)

The idea that considerations of subunit symmetry alone dictate the hand of a helical structure is obviously a very appealing one. It is of course only an approximation to the truth – which is that helix hand is fixed physico-chemically through detailed patterns of atomic contacts. This is revealed in the filamentous viruses and in the single turn of left-handed helix in thermolysin. And it is worth adding that Bradbury et al.[5] showed that the α-helical hand of poly-β-benzyl-L- aspartate (which is not a feature of proteins) is left-handed, not right.

A challenge to the staircase analogy is presented by synthetic polypeptides with alternating L and D amino acids[37]. Alternation is also a feature of the antibiotic, gramicidin A[46]. This antibiotic, isolated from *Bacillus brevis* acts by forming channels through biological membranes. Both papers conclude that these alternating polypeptides have helical conformations and indeed Heitz et al. suggest that the conformation is very similar to α-helix. This is a puzzling suggestion especially since they do not say whether it is a left- or right-handed helix and one cannot be favoured over the other on energetic grounds. So do both hands exist? Is there here an example of true helical enantiomorphism? Despite the claims of theorists, the odd idiosyncratic exception and some explanatory difficulties, the message from molecular biology is clear, however. Ambidextrousness is not a feature of biology at the molecular level.

Phyllotaxis: helix hand decided at random

Ambidextrousness, is however, a very decided feature of the structures of organs and whole animals and plants (although not necessarily always a common one). For instance typically, the relationship of any one leaf on a twig to the next is that of a constant screw displacement. If this displacement is represented as a fraction of the twig's circumference, the numerator and the demoninator are successive integers of the celebrated Fibonacci series....

1,1,2,3,5,8,13,21 ....

(Where each integer is the sum of the two immediately before it.) .... or if the leaves are traced on a helix of the opposite hand alternate integers of the series. Thus the beech exhibits what is known as $^{2}/_{3}$ (or $^{1}/_{3}$) phyllotaxis (literally leaf arrangement) the oak $^{3}/_{5}$ (or $^{2}/_{5}$) and the pear $^{5}/_{8}$ ($^{3}/_{8}$) – and so on.

"The Fibonacci pattern seems to be a robust and stable mathematical phenomenon, a finding that goes some way to explaining its widespread occurrence throughout the plant kingdom"[54]. For example the same pattern manifests itself in the spiral arrangement of florets in the head of sunflowers, in that of the scales on pine cones and in the arrangement of leaf bases along helical paths called parastichies on the surfaces of pineapples and the 'trunks' of palm trees (fig. 6). These arrangements exist as right and left mirror images of each other.

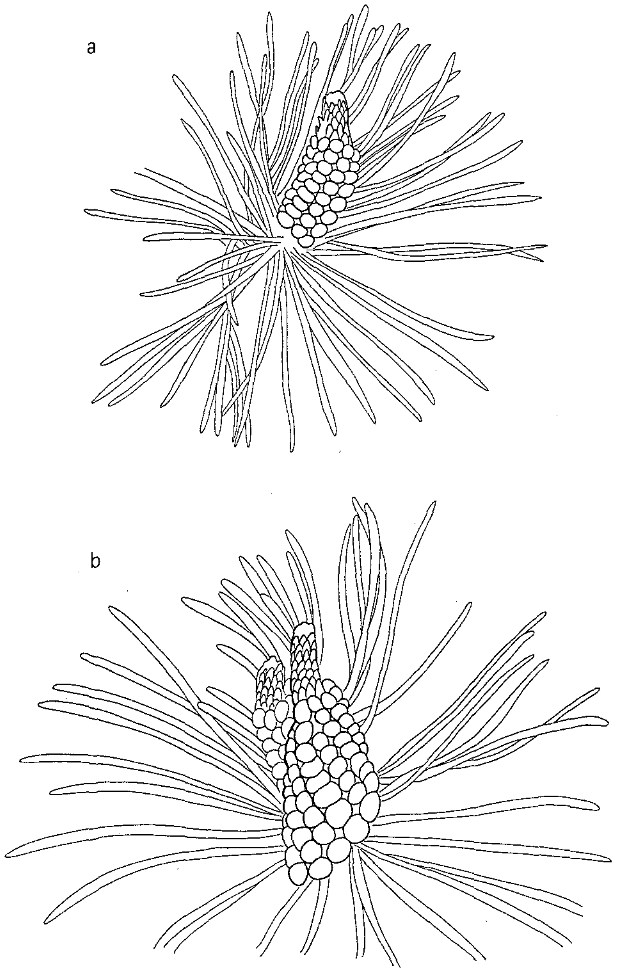

Figure 6. *a* Right-handed and *b* left-handed male cones from *P. mugo*. (Drawn by Joe Brock.)

That phyllotactic hand is determined entirely at random and is under no sort of genetic control has been shown very compellingly by Davis[22] for species of palm trees. He examined more than 50,000 speciments of *Cocos nucifera*, the coconut palm, from 36 countries finding that they were distributed evenly between left and right hands. He also undertook breeding experiments. When right-handed seed parents and right-handed pollen parents were bred under controlled conditions, half the progeny were right-handed and half left. Exactly the same was true of the progeny of each of the other possible crosses LxL, RxL and LxR.

(Not all palms exhibit spiral phyllotaxis. There are neutral palms where the leaves are arranged in vertical rows: two rows in *Wallichia disticha*; three in *Neodypesis decarii*, and examples with greater numbers of rows – orthostichies – are known. Occasionally variations of these are found where the orthostichies are twisted into helices.)

Rijven[63] investigated the mechanisms underlying the random allocation of handedness in spiral phyllotaxis. He found using fenugreek seedlings (*Trigonella foenum – graecum* L.) that the third leaf from the apex can occupy alternative sites – to the right and left of the first leaf and that it is here that left/right dissymmetry is introduced. He examined a few hundred plants and found that within the limits set by the statistics the right and left positions were occupied at random. However in a small proportion of seedlings both positions were occupied resulting in symmetrical rather than dissymmetrical plants. A randomly allocated enantiomorphism is also a feature of the root of the water fern *Azolla* which was the subject of a careful investigation by Gunning et al.[36].

The examples I have talked about here are all angiosperms – flowering plants. Gymnosperms seem to behave rather differently. For instance right- and left-handed pine cones are found on the same tree (fig. 6). What recorded evidence there is supports the idea that right and left cones are found in the same proportions in trees of the same species and from year to year in the same tree. However the ratio is not unity but varies among species: close to 1.2 for *Pinus austriaca* to 2.4 for *P. laricio*. This rather suggests a genetic component to the control of handedness but in what way it is exercised, I have been unable to discover.

Situs inversus: loss of genetic control

A vertebrate's bilaterally symmetrical appearance is misleading. Even outwardly, people's left and right halves are rather less of mirror images than is often presumed[59]. Inwardly they are even less symmetrical. Since the viscera are asymmetric the possibility exists for individuals, including individual human beings, to be born with their

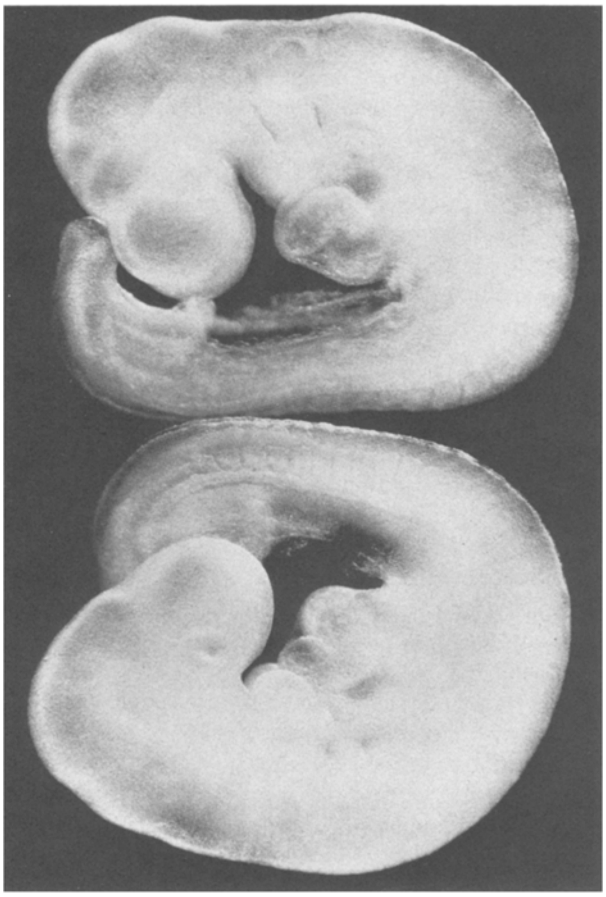

Figure 7. Early rat (and other) embryos have a decided helical appearance. Left- and right-handed rat embryos which would give rise to situs solitus and situs inversus (Courtesy of Dr Nigel Brown).

normal visceral symmetry reversed. And indeed such individuals do exist although the phenomenon – known as situs inversus – is rather rare in human beings, occurring in about 1 in 10,000 adults[47]. The normal condition of unreversed symmetry is known as situs solitus (fig. 7). A number of studies of the genetics of situs inversus in mice have been made, for instance by Hummel and Chapman[39] and by Layton[47] which produced a rather striking finding – namely that situs inversus is the result of an autosomal recessive gene (the gene is designated iv) but that even among homozygotes, the incidence of the condition never exceeds 50%. This led Layton to propose that the normal allele at the iv locus specifies the normal symmetry but that absence of the normal allele allows symmetry to be assigned quite randomly. McManus[52] has pointed out that an exactly analogous mechanism would account for the inheritance of the relatively rare left-handedness in an otherwise right-handed human population. (Right- and left-handedness are to be taken literally in this context.)

Gastropods: helix hand assigned genetically

The conchospirals of gastropods are true helices (see the section on geometry) and are almost always right-handed – although not quite. Most species are right-handed, (dextral), although a number of exceptions are known. The entire family of the Clausiliidae is predominently left-handed. Some species of *Partula* are left-handed. Both of these are land snails. Among left-handed (sinistral) marine gastropod species are a number belonging to the family, Triphoridae, the sinistral miniature horn shells. Occasional sinistral species occur in otherwise dextral families and as Gould[34] points out the idiosyncracy is marked in their name. *Neptunia contraria* and *Busycon perversum* are a couple of instances. However in addition to the occasional left-handed species or even larger groups some right-handed species also occasionally contain left-handed individuals. Both *Limnaea peregra* (the pond snail) and *Helix aspersa* (fig. 9), display this phenomenon (see also figure 8) (and sinistral species sometimes contain dextral individuals). The rarity of left-handed conchospirals has attracted attention at least since Aristotle and no doubt from before him[69] and they are regarded with varying degrees of curiosity, astonishment and of course scientific interest – which sees in their rarity two facets of an evolutionary puzzle; what devel-

Figure 8. The Hindu God, Vishnu is traditionally represented holding the shell of *Turbinella*. The shell is always shown left-handed. In fact only five examples of the left-handed form are known to exist[35]. (Courtesy of the Hulton picture library).

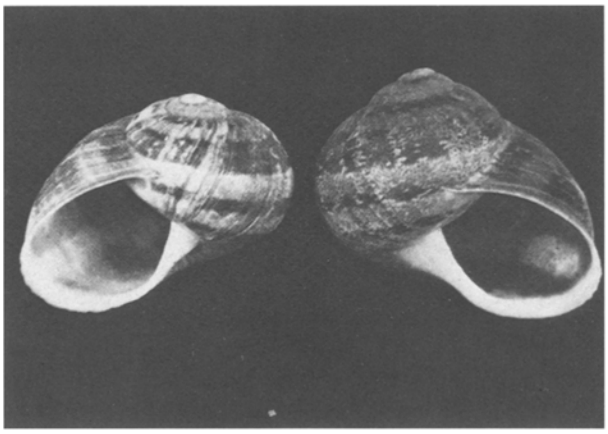

Figure 9. *Helix aspersa*, the common British snail. Left-handed and right-handed (normal) mutual mirror image shells.

opmental mechanisms control, and what evolutionary mechanisms maintain, the rarity of left-handedness.

For completeness it is worth noting[70] that this preponderance of one type of symmetry contrasts with the approximately equal numbers of right- and left-handed conispirally coiled fossil nautiloid and ammonoid cephalopods.

I hope that it is apparent even from the brief discussion of helical geometry early in this note that in principle at least a left-handed gastropod might evolve in two quite distinct ways.

The way I am mainly concerned with in this article is a reversal in the sense of coiling in a right-handed shell producing a mirror image of the shell and the anatomy of the animal it contains. This phenomenon appears to be under genetic control. Handedness in snails is a simple Mendelian characteristic although with the interesting feature of being an instance of maternal inheritance – the hand of coiling is determined not by the individual snails own genes but by that of its mother[67].

However an unhanded planispiral shell might over time evolve with equal facility – or difficulty – into a right-handed or left-handed conispiral shell depending on the direction in which the axis of coiling is pulled. Interestingly, something similar can also happen in an abnormal development of a conispiral shell. The mature adult shell is built on the larval proto-conch. Occasionally it builds in the wrong direction resulting in a shell of the wrong hand. One of the ways this 'hyperstrophy' or 'ultradextrality' is recognised is that the shell and the animal it contains have opposing hands. Normally a right-handed shell contains a right-handed animal and a left-handed variation on it, a corresponding left-handed animal. Hyperstrophy is defined, illustrated and discussed all very well by Knight[44]; see also Robertson and Merrill[64].

In considering the two mechanisms – developmental and evolutionary – that keep left-handedness so rare, I would like to start with the evolutionary one. On the face of it it seems inconceivable that evolution could discriminate in favour of a right-handed conchospiral and against its left-handed mirror image. What possible advantage could a right-handed sense of twist confer over a left-handed sense? In fact of course there is no reason to suppose that the major morphological change of reversing the sense of twist leaves everything else exactly the same. In this case evolution might act not against the left-handedness itself but against concomitant changes. Gould et al.[35] examined 28 physical characteristics of the only five available sinistral specimens of *Cerion* (the peanut shell) and compared them with the mean values for samples of dextral *Cerion*. They concluded that in respect of some groups of features there are indeed systematic structural differences between right- and left-handed individuals including a distortion of the axis of coiling.

However in *Partula*, Johnson[41] says that shell shape and screw sense are evolving separately and offers the alternative hypothesis that evolution is not acting against left-handedness itself but against its relative rarity. Indeed rarity for variation in handedness is more striking even than that for sinistrality.

How could evolution discriminate against a rare left-handed form in a predominantly right-handed population and vice versa. Sturtevant[67] may have made the significant observation here when he pointed out – referring to left-handedness in *Limnaea peregra* – that the right- and left-handed forms might not be able to mate with one another. An individual of the rarer form will have relative difficulty in finding a mate of the same hand thus keeping the rare form rare or creating separate breeding populations which under some circumstances might become geographically distinct. This is true of species of *Partula* on the islands of Tahiti and Morea where they are found.

This argument does not of course explain the predominance of right-handed forms but it does link the rarity of left-handed species with the rarity of left-handed individuals in predominantly right-handed populations and suggests how the imbalance could be maintained. Johnson[40] has managed to show that Sturtevant's supposition is essentially correct, snails of opposing hands mating relatively rarely. But he has also observed that nature, not content with making mating difficult contrives that the fruits of any unions that do take place are less numerous in the rarer than the common form. How this finding relates to the greater mortality after birth of the rare sinistral as opposed to the much commoner dextral form in *Melantho* observed by Fischer and Bouvier[28] is not clear.

That the mirror dissymmetry itself, rather than a collection of small defects associated with the left-handed reflection plays an important part in evolution is a very appealing idea. And indeed, a switch of hand may on occasion be advantageous as Clarke and Murray[11] pointed out *in P. suturalis*. It may prevent mating with a different though closely related snail with the same sense of twist. Advantage or disadvantage, what about the mechanism underlying the striking morphological change of mirror reflection. Gastropods exhibit spiral cleavage in their early development (fig. 10); after the egg has become 4-celled each cell splits into a larger daughter lying towards the lower pole and the smaller one towards the upper pole of the egg. The upper cell does not lie directly over the lower one but it is displaced in a clockwise or anticlockwise direction. Divisions always follow the order: clockwise – anticlockwise – clockwise in a right-handed animal and the opposite order in a left-handed animal. The female gastropod produces eggs with a preformed handedness but this is not apparent until the third cleavage. (A good review of spiralian development is Costello and Henley[17].)

Neither phyllotaxis, with its random assignment of hand, nor situs solitus and inversus where hand is usually fixed genetically and where only loss of control leads to random allocation of hand seem to have much to tell us about gastropods. Despite 70 years of research, gastropod genetics remain somewhat enigmatic in two important respects. It is well established that left-handedness originates in an autosomal recessive gene[4,29]. Delayed maternal inheritance[67] implies that all the offspring of a female snail irrespective of their own genotypes, should possess the same hand. This turns out not to be true. Some litters possess some snails of opposing hand. Layton's[47] model for situs would go some way towards explaining this – a mother homozygous for the recessive gene would produce litters on average possessing half right- and half left-handed progeny. However one of the sinistral lines in Boycott et al.'s[4] experiment gave only 8 dextral snails out of 20,000. Albino inheritance in the same snails was inherited with normal Mendelian behaviour. Freeman and Lundelius[29] have developed a crossover model to explain the appearance of the anomalous wrong-handed individuals.

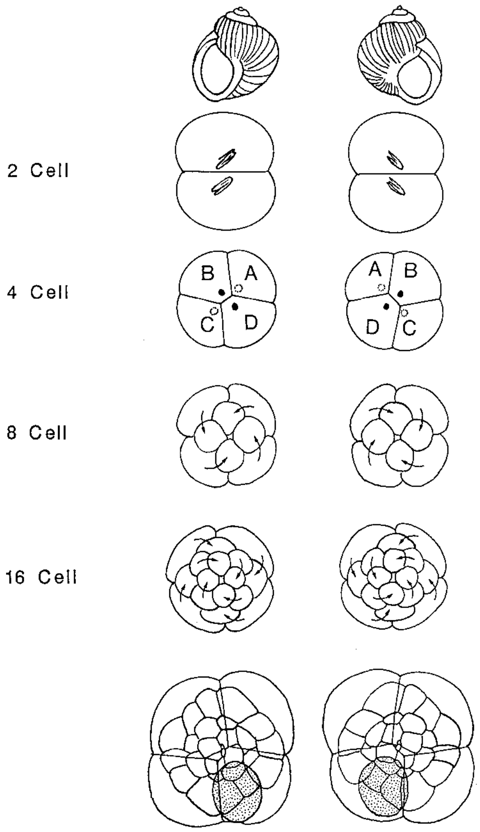

Figure 10. Spiralian development: the effect of 'spiral' cleavage on snail twisting. Sinistrals are on the left, dextrals on the right. Oblique cleavage plane set by mitotic spindles gives rise to oblique furrows in the four-cell stage. For dextrals the third division is anti-clockwise, the fourth clockwise and so on. Bottom drawings shows the large mesoderm cell (stippled). On the right in sinistrals, left in dextrals. This cell develops into the muscle for torsional symmetry[59].

The real problem with the gastropod genetics however is this. A recessive gene usually represents a loss of function in the normal gene product. One reason why a priori Layton's[47] model is rather plausible for example and consistent with Davis' findings for palm trees. But for gastropods loss of gene function does not lead to the expected and comprehensible replacement of a deterministic mechanism by a stochastic one but by another deterministic one. Indeed it looks as though the natural hand of twisting is to the left which a gene changes to right-handed – with the opposite being true of left-handed species. This is a curious phenomenon and it seems unlikely to be resolved without some sort of understanding of the molecular basis of spiralian development. What is the nature of the protein for which the right-handed gene codes? This is an excellent question to be attacked by modern methods of molecular genetics which could identify and characterise the gene.

Hand determined non-genetically

In gastropods right/left-handedness is controlled by a gene product stored in the cytoplasm of the oocyte. Cytoplasm can often be important in development. Among the ciliates cytoplasmic inheritance is common. Usually the effects can be traced to nucleic acids, not free in the cytoplasm however but packed in the organelles for example[56]. Recently Nelsen et al.[58] have proposed that aspects of cellular handedness – as revealed in the arrangement of cilia of the cells surface – do not originate in genes and are examples therefore of true cytoplasmic inheritance. They suggest that a system of positional information within the cytoplasm may be inherited directly – not through a gene code. The difference between the ciliates and the organisms that have been discussed in the review so far is of course that ciliates are acellular, i.e. single-celled whereas left- and right-handedness in gastropods or in phyllotaxis is a property of different arrangements of large numbers of cells.

In fact a wide variety of suggestions have been made for factors other than genes that influence handedness. Temperature for example. A considerable body of at least circumstantial evidence supports the idea that water temperature has determined the hand of the tests (shells) of some species of foraminiferans. Left-handedness is associated with low, and right-handedness with higher temperatures, in *Globigerina truncatulinoides* and *G. pachyderma*. This is so definite an effect that it is used by oil companies looking for clues as to where to prospect. A temperature induced switch of hand in the highly organised, helical multicellular structure of *Bacillus subtilis* has been studied by Favre et al.[26]. Coakley and Brown[12] have proposed that differences in tissues of oxygen concentration may influence hand in embryonic development. Situs inversus can be induced simply by surgical manipulation or by injecting tissue homogenates[47].

An apology

I am rather conscious of the telegraphic style of this review – that I have made the subject appear both much smaller than it is in reality and much simpler. Many examples well worth including and discussing have been left out – climbing plants and the phenomenon of nutation, the helicoids of plant cell walls and insect cuticles, helical bacteria, worm cuticles and animal body walls to give some examples.

It is also true that the choice open to organisms is not limited to left and right hands but sometimes includes the option of straightness as I mentioned in discussing palm trees – and this is true of trees more generally[66], and at the other end of the scale some families of bacteria. In gastropods and animals related to them, and option is for planispiral forms – the ammonites for example or *Planorbis*[45]. I am sorry to have left them out but to have included them might have laid me open to the criticism once levelled by James Thurber at a book, "this book tells me more about penguins than I want to know". I hope I have said enough about left- and right-handedness – but not too much.

Appendix 1

Crystallographic screw axes

Somewhat related to the problem of the symmetry adopted by the big biological polymers is that of what dictates the space group symmetries of crystal lattices. 230 space groups are possible. 65 of these are 'homochiral' and give rise to enantiomorphic structures – they are composed only and completely of direct isometries but not the opposite isometries – reflections, rotations, rotatory reflections, inversions and glide reflections. These are the groups in which assemblies of homochiral molecules are obliged to crystallize.

The most interesting subset of the 65 are the 11 mutual mirror image pairs, one of each pair containing right-handed screw displacements, the other reflected left-handed screw displacements. When mutual mirror image pairs of molecules crystallise in the mutual mirror image pairs of space groups, does the hand of monomer force the space group's hand? Presumably the answer must be yes. More interestingly do all the L-amino acids crystallize in space groups with the same screw sense? An examination of 665 molecular species in the Cambridge Crystallographic Data Centre crystallising in these 22 space groups failed to identify a single example where the lattice symmetry had been obtained for both enantiomers of an optically active molecule. However, the analysis did throw up a rather striking fact. Space groups with right-handed screw symmetries are nearly three times as common as their left-handed mirror image counterparts. (table 2). The statistical significance of this finding seems overwhelming. What it means – whether it is an artefact of some sort or whether it has real physical significance – I have no idea.

Table 2. Distribution of 665 structures in the Cambridge Crystallography Data Centre among the 22 enantiomorphic space groups that exist as 11 pairs, each pair possessing as symmetry elements, mutually reflected nonsuperposable screw axes. The preponderance of right screw axes over left is almost three to one. This is in statistical terms very unlikely indeed ($X^2 = 155$).

	Right-hand screw		Left-hand screw	
Crystal class	Space group	Frequency	Space group	Frequency
Tetragonal	$P4_1$	100	$P4_3$	19
	$P4_122$	8	$P4_322$	2
	$P4_12_12$	203	$P4_32_12$	71
Trigonal and hexagonal	$P3_112$	1	$P3_212$	2
	$P3_121$	61	$P3_221$	20
	$P3_1$	47	$P3_2$	24
	$P6_1$	41	$P6_5$	25
	$P6_2$	4	$P6_4$	2
	$P6_122$	18	$P6_522$	3
	$P6_211$	7	$P6_411$	2
Cubic	$P4_132$	3	$P4_332$	2
Total		493		172

Appendix 2

A note on nomenclature

It is sensible to call a pair of mirror helices left- and right-handed but what rationale is there behind the choice of which to call left and which right? The design of the human hand, wrist and forearm favours right-handed screws and cork screws – using the above convention – for right-handed people, which provides a perfectly good basis for choice. (Although Cook[16] pointed out that some botanists proposed to call a left-handed twist in a rope right-handed because it resulted from the twist given by someone right-handed, and to use the same convention for plants.) Electromagnetism employs the left- and right-handed rules which provides a rather different basis for choice but happily one with the same result.

Chemistry of course prefers Greek to the plain English 'handedness' and uses either chirality (chiros = hand) or enantiomorphism (literally, possessing contrary forms). Pasteur coined 'dissymmetrie' for this property and 'dissymmetric' is sometimes used in English to describe a pair of non-superposable mutual mirror images.

A convention often used is to describe a left-handed helix as clockwise and a right-handed helix as counter clockwise. These terms are used, for example, in bacteriology[2]. Yet other conventions involve the terms S and Z. Traditionally right-handed rope is referred to as Z-laid, and left-handed as S-laid (see the section on molecular helices). The S and Z terminology has also been used by plant morphologists in describing helical arrangements of cellulose fibres in plant cell walls[30] – the Z and S layers. However, the distinction between these layers is rather blurred and so are the terms therefore. The terms S and Z are also used in astronomy but in the opposite sense in referring respectively to (apparently i.e. seen from the earth) anti-clockwise and clockwise rotating spiral galaxies. Left-handed DNA is of course called Z-DNA but here Z stands for Zig-Zag[23] and has nothing explicitly to do with the *hand* of the helix.

In describing the conchospirals of shells a right-handed shell has sometimes been called leiotropic, i.e. left turning, because in ascending the spiral the trajectory is always turning to the left[16] and a left-handed spiral described as dexiotropic. The more explicit dextral for right-handed and sinistral for left-handed are the terms usually adopted now. The variation on these terms, dextrorse and sinistrorse, is found in plant morphology. In chemistry, the absolute configuration of enantiomorphic molecules may be designated rectus and sinister.

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Research Articles

Morphological integration in the cranium during anuran metamorphosis

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Summary. We examined the role of thyroid hormone in mediating morphological integration between cranial cartilage and bone during anuran metamorphosis. Exogenous T_3 applied to premetamorphic tadpoles (*Bombina orientalis*) via intracranial implants of plastic micropellets precociously induced typical metamorphic changes in both tissues, but also dissociated the relative timing of developmental events between them. Morphological integration between the two primary cranial tissues is achieved in part by each tissue responding independently to endocrine factors and does not reflect a tight developmental coupling between them.
Key words. Morphological integration; skull; Anura; thyroid hormone; metamorphosis.

Mechanisms of morphological integration are among the most important, albeit poorly understood, organizational processes in development[1–7]. An outstanding example of morphological integration is the suite of changes that comprise cranial metamorphosis in anuran amphibians. During metamorphosis in these vertebrates, the cartilaginous larval skull is dramatically transformed by a combination of proliferation and resorption of existing cartilages and the formation of new ones; bone, which predominates in the adult skull, also appears for the first time. Moreover, the events by which cartilaginous tissues transform and bony tissues differentiate occur in a precise temporal sequence that achieves a high degree of integration both among components of a given tissue type and between bone and cartilage.

Endocrine factors, particularly thyroid hormone (TH), play a predominant role in mediating morphological changes during amphibian metamorphosis[8]. Thyroid hormone is also known to affect the differentiation, growth, and remodeling of skeletal tissues in both am-