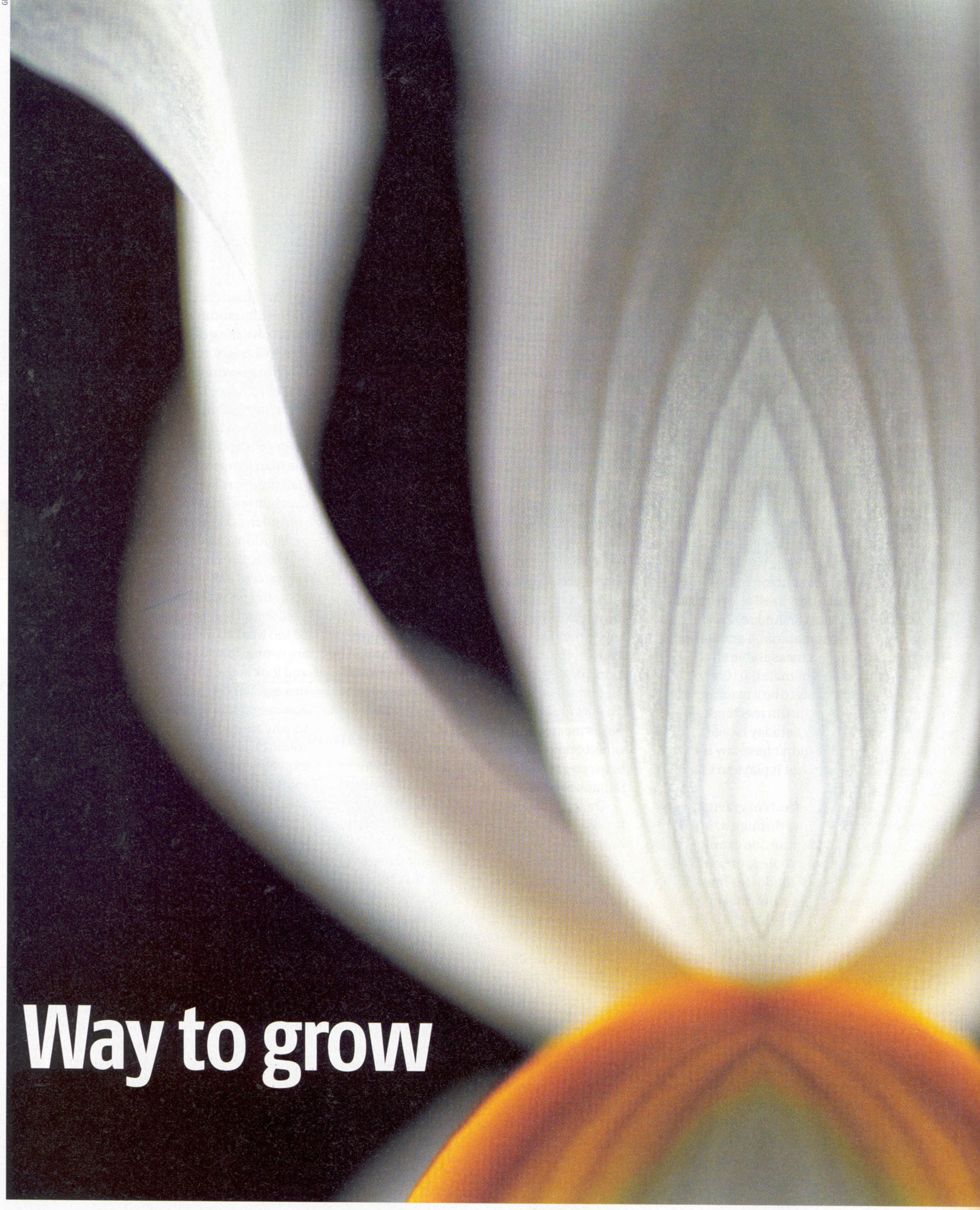

Way to grow

How do plants and animals translate their genes into the graceful curves of leaf and limb? Developmental biologist **Enrico Coen** is uncovering the elegant simplicity behind complex forms

"ALL beautiful forms must be composed of curves," declared Victorian art critic John Ruskin, "since there is hardly any common natural form in which it is possible to discover a straight line." Whether or not you agree with Ruskin's views on beauty, it is true that life seems to be dominated by curves: the curving outline of a leaf, a butterfly wing, a flower or the human body. A world of only straight edges and flat surfaces would seem alien to us.

Yet we know very little about how living creatures produce these curves. It is fairly easy to see how artists draw their curves, simply by examining their brush strokes. One of Ruskin's contemporaries, William Morris, was famous for his sweeping designs that drew inspiration from natural forms (see Picture). But finding out how flowers or insects draw their curves is much harder, for here the artists are not painters, but genes.

Even though we have sequenced the entire genomes of several plants and animals, we don't understand how these linear strings of information produce the complex three-dimensional living forms we see around us. Developmental biologists have learned a lot about how the patterns of gene activity in a developing embryo are established, yet remain pretty much in the dark about how these patterns are connected to building shapes. The problem requires an understanding of geometry as well as genetics, and it is one that several labs, including my own, are beginning to tackle. Central to the question of how genes control shape is how they influence the way tissues curve and bend as they grow.

We know that genes are somehow involved because mutations in genes can change the shape of a structure like a leaf, as a trip to the salad section of your supermarket will show. The differences between flat and crinkly varieties of lettuce reflect differences in their genes. Surprisingly, it only takes changes in one or two genes to produce dramatically different leaf shapes. In my lab at the John Innes Centre in Norwich, for example, we have found that the loss of a single gene called *CIN* causes the normally flat leaves of a snapdragon plant to become ruffled. But how can such simple changes influence the complex curvature of leaves and flowers?

We can begin to tackle this problem by thinking about how plants go about building themselves. For one thing, we know that organisms generate their curves in a very different way to a human artist. If we want to produce a curved leaf shape, we might paint some outlines on a canvas. The canvas provides a fixed space to draw on. A leaf, on the other hand, forms its shape by the way it grows. As a material structure that extends in space, a leaf is more like the canvas than the lines that are drawn upon it. But it is a strange canvas, one that can grow and change its shape. A leaf creates its own space as it develops, and it is the shape of this space that defines the outline of the leaf. This presents a challenge because we need to deal with a spatial framework that is continually growing and changing rather than having one of fixed size to which we can refer.

The next problem is to understand how this living canvas extends itself. We know that there are basically two ways for things to grow. The first is accretion – the addition of material to the outer edges of a structure. This is the type of growth we are most familiar with in everyday life. When builders build a house, they add bricks onto the ones already there, extending the boundaries of the building either upwards or horizontally. Shells grow in this way, forming by continual deposition of ▶

It is fairly easy to see how Victorian artist William Morris drew his curves (below). But how do plants do it?

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calcified material at the shell's mouth. The rate of deposition at the edge usually varies around the opening, resulting in curved shapes.

But this is not the way a leaf or limb grows. Structures like these develop by the growth and division of the cells within them, rather than by adding material to their boundaries. This means growth can be more broadly distributed through the structure. If a wall could grow by means of each brick within it gradually getting bigger and then dividing to give two bricks, the growth would take place across all regions of the wall at once rather than being limited to its boundaries. For this to work, the bricks would have to be flexible so they could change size and stretch or contract to accommodate variations in the growth of other bricks.

Flexibility is not a problem for animal cells because their surrounding membranes are supple. They can also move and slide past each other if necessary. But plant cells are trapped by the cell walls that enclose them, so the flexibility has to come from the walls extending. From a biologist's point of view, this constraint on plant cells makes it easier to study shape, because there's no need to worry about cells moving around.

What sort of shapes might you expect to emerge from plant growth? It helps to think about two processes – one that promotes straightness and another that introduces curves. If all the cells of a structure tend to grow and divide uniformly in the same direction, then the structure will gradually elongate in a straight line along that preferred direction of growth. The straightness of a plant stem, for example, reflects the fact that the main direction in which its cells grow lies parallel to the axis of the stem.

We don't yet know precisely how growth is oriented in a particular direction. One possibility is that there is a concentration gradient of some special molecule along the stem, or a flow of some molecule in a particular direction. Cells could then align their growth along the gradient or direction of flow and so keep their growth in a straight line.

On the other hand, curves appear when the rate or direction of growth is not uniformly distributed throughout the structure. Suppose we start with a rectangular sheet of cells marked with a grid of squares so that we can keep track of its regions. If every cell grows at the same rate and equally in all directions, then the sheet will simply get larger without changing shape. But now imagine that the rate of cell growth in our initial rectangular sheet increases steadily from one end of the rectangle to the other (see Diagram, opposite).

Some cells will now try to grow slightly faster than their neighbours and the conflict will result in the lines of the grid gradually bending, with the regions rotating relative to each other. It is the continuity of the sheet – the connections within and between the cells – that forces the regions to curve and turn to accommodate each other's growth.

In this case the curving occurs within one plane and the structure remains flat, but it is also possible to distort a flat sheet to produce ripples or waves. Suppose the cells in our original rectangular sheet still grow equally in all directions, but now the rate or extent of growth gradually increases towards the edges of the rectangle (see Diagram). The only way to accommodate this extra growth without the sheet tearing is for the edges to bend or ripple. This is the mechanism behind the wavy edges of leaves like those of sea kale, which cannot be flattened without producing folds or overlaps.

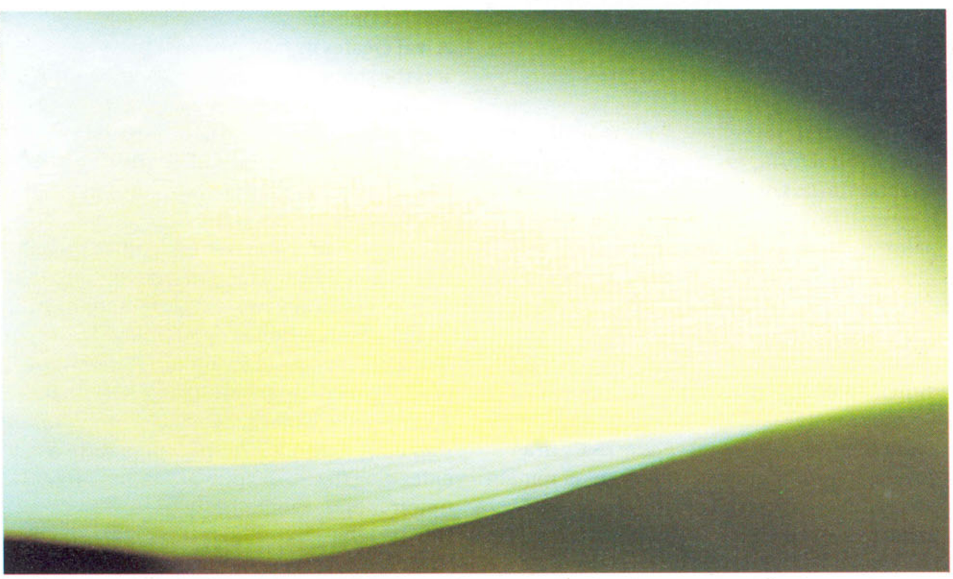
So shape and curvature depend on carefully controlling relative growth rates in different parts of a tissue. To understand how genes might achieve this, Utpal Nath, Brian Crawford, Rosemary Carpenter and I, working at the John Innes Centre, decided to study mutant forms of snapdragon (*Antirrhinum majus*) that lack the *CIN* gene. Leaves of normal snapdragon plants have a simple outline and can be flattened without introducing folds, but removing the *CIN* gene generates undulating edges that cannot be easily flattened. It is as if the *CIN* gene is needed to keep the leaves flat, so the obvious question is, what does it do?

We found that *CIN* influences the way in which leaves stop growing (*Science*, vol 299, p 1404). Leaves need to stop growing after they have reached a certain size, and in many plants, this arrest does not happen simultaneously throughout the leaf, but occurs progressively. First the tip of the leaf stops growing, and then the arrest gradually spreads towards the base until the whole leaf has stopped. By the time you see a leaf starting to emerge from a bud, the cells in the leaf tip have already stopped dividing and the process of arrest is on its way.

In plants that lack the *CIN* gene, we found that leaves do not stop growing in the normal way: the arrest proceeds much more slowly, particularly along the margins of the leaf, so these regions keep growing for longer. It is this extra growth at the margins that creates the undulating edge.

CIN makes a regulatory protein that can switch genes on or off. It probably works by controlling the activity of genes involved in arresting cell growth and division, particularly in the margins of the leaf. Removing *CIN* allows cell division and growth to continue for longer than normal, giving rise to wavy leaves.

We tend to take the flatness of a leaf for granted, but clearly plants go to considerable lengths to produce this result. Flatness



Different rates of growth within a structure can result in twists and curves – such as a curved leaf (above) or the curling petals of a snapdragon flower (right)

requires finely regulated growth so that one region does not grow too much relative to another. Genes like *CIN* play a role in ensuring this happens correctly, reining in life's inherent tendency towards curviness.

Petal power

So far we have considered straightness or curving separately. But life is rarely that simple. In many cases, both of these growth processes may be going on at the same time. There might be preferred growth in a particular direction, while at the same time different regions might grow to different extents or at different rates, creating curves. The resulting shape would be a sort of hybrid of the straight and the wiggly. However, working out what sort of shapes to expect from this is not so easy because the interaction of these different processes in a growing structure is far from simple. To solve this problem, some biologists are turning to computers.

That was the approach behind a recent study carried out by research student Anne-Gaelle Rolland-Lagan, working with myself and Andrew Bangham at the University of East Anglia in Norwich (*Nature*, vol 422, p 161). We wanted to work out how the petals of a snapdragon flower grow. Snapdragon flowers have a complicated shape. The petals are partly joined together to form a tube with a closed "mouth" that bees can open by prising the upper and lower petals apart. Finding out how the petals develop in the young flower bud is very difficult because they are tiny and hard to monitor at this stage. So we found a way of marking cells in the young flower bud that would let us follow their development.

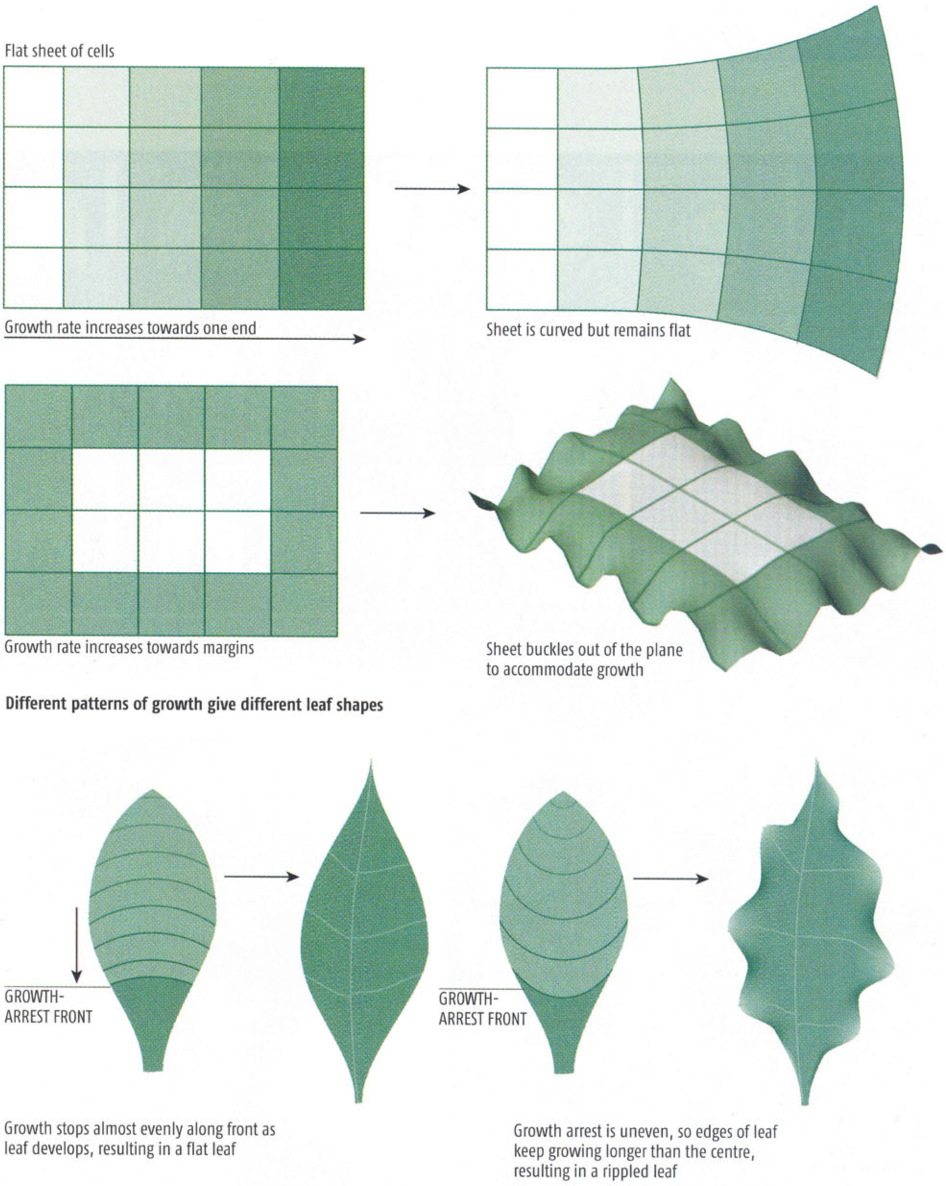
To see how it works, imagine you put a series of small circular tattoos in various places on a young baby's arm. As the baby grows up, the tattoos will grow with the arm, changing in shape and size according to how the arm grows. By looking at the final shapes of the tattoos on the adult and comparing them to the initial shapes you might then be able to say something about how the arm grew between birth and adulthood.

We used a similar principle, but instead of applying tattoos, we induced a genetic change in single cells so that their descendants became red when the flower reached maturity. The resulting flowers have a spotty or stripy appearance. Unlike the tattoos, though, we could not observe the spots when they were first induced, nor could we control their exact starting location. Nevertheless, we could use a computer to work out how the petal grew by analysing and comparing lots of spots on mature flowers that had been induced at various times.

This gave us a model for how the different regions of a snapdragon petal grow to form the final shape. Simulations using this model indicate that the shape of the petal depends on

GROWING CURVES

Curved shapes result when one part of a tissue grows faster than another



two things going on in parallel. On the one hand, there seems to be a signal that promotes growth along the direction running from the base to the tip of the flower. On the other, different regions of the petal can grow to different extents along this direction, causing some to curve relative to others.

The lesson is that even though a final shape can look quite complicated, the underlying growth patterns may be relatively simple. It is the combination of directed and differential growth that allows apparent complexity to emerge. With these simple principles in hand, it should be possible to develop models of how plants, or even animals, build their shapes. It should also make tracking down the genes involved much easier.

But even though some of life's growth processes may be simpler than they seem at first, we are still very far from being able to predict an organism's shape from the sequence of letters in its DNA. Enormous gaps remain in our knowledge of how genes influence the properties of cells, and how cells signal and interact with each other within growing frameworks. Only when these blanks have been filled in, perhaps by collaborations between biologists and computer scientists, can we hope to fully understand how living things create the beautiful forms that delight and inspire us. ●

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