

REVIEW

On genes and form

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ABSTRACT

The mechanisms by which organisms acquire their sizes and shapes through growth was a major focus of D'Arcy Thompson's book *On Growth and Form*. By applying mathematical and physical principles to a range of biological forms, Thompson achieved fresh insights, such as the notion that diverse biological shapes could be related through simple deformations of a coordinate system. However, Thompson considered genetics to lie outside the scope of his work, even though genetics was a growing discipline at the time the book was published. Here, we review how recent advances in cell, developmental, evolutionary and computational biology allow Thompson's ideas to be integrated with genes and the processes they influence to provide a deeper understanding of growth and morphogenesis. We consider how genes interact with subcellular-, cellular- and tissue-level processes in plants to yield patterns of growth that underlie the developmental and evolutionary shape transformations Thompson so eloquently described.

KEY WORDS: D'Arcy Thompson, Morphogenesis, Plant growth

Introduction

In the preface to his book *On Growth and Form*, D'Arcy Thompson described how writing both the first edition (Thompson, 1917) and the revised edition (Thompson, 1942) gave him solace and occupation during the two world wars. Those periods also coincided with striking developments in genetics. During the first period, Mendelian genetics was in conflict with Darwinian natural selection (Provine, 1971); the discrete particles of heredity were seen to be at odds with the idea of continuous variation and gradual adaptation. This conflict was largely resolved during the 1930s, with the development of population genetics. By the time of the Second World War, genetics and the theory of natural selection had been fully integrated within evolutionary biology, to provide what became known as *The Modern Synthesis*, a term coined in 1942 by Julian Huxley (Huxley, 1942).

Yet in the field of morphogenesis, divisions persisted. Thompson expressed scepticism about the relevance of Darwinian explanations for understanding growth and form. In his view, Darwinians too often resorted to teleological explanations, accounting for patterns such as hexagonal honeycombs, spots on a feather or the twisting stems of a climbing plant in terms of adaptations. For Thompson, the true cause of these phenomena lay in physical processes, such as the tension in warm bee wax causing hexagonal geometry, chemico-physical diffusion causing pigment patterns and differential growth causing the twist in a stem. The solutions lay in mathematics and physics, not in function and adaptation.

Thompson wrote less about genetics, though it is occasionally mentioned in the 1942 edition. There he says that he leaves genetics

aside 'not because I doubt for a moment the facts nor dispute the hypotheses nor decry the importance of one or other; but because we are so much in the dark as to the mysterious field of force in which the chromosomes lie, far from the visible horizon of physical science, that the matter lies (for the present) beyond the range of problems which this book professes to discuss' (p. 341, Thompson, 1942). Much of the darkness and mystery Thompson refers to was lifted in the second half of the 20th century, as the nature of genes and their mechanisms of action became clear. Nevertheless, the link between gene activity and the generation of form remained obscure, largely because of difficulties in determining growth patterns and relating them to physico-chemical mechanisms. However, recent advances in imaging and computational modelling (Sharpe, 2017) have begun to address these issues. We are now at a time of synthesis, when the insights of Thompson can be integrated with findings drawn from genetics, cell biology, development, evolution, materials science and computational biology, to give a deeper understanding of the generation of form. Here, we review some of the concepts that have emerged from these findings, seen through the eyes of plant developmental biology, using the growth of planar tissue shapes as an example (for reviews of non-planar forms, see Prusinkiewicz and Runions, 2012; Whitewoods and Coen, 2017). We begin with the nature of growth (Goriely, 2017).

The nature of growth

'It is obvious that the form of an organism is determined by its rate of growth in various directions; hence rate of growth needs to be studied as a preliminary to the theoretical study of form' (p. 79, Thompson, 1942).

The importance of rates and directions of growth for the generation of form were obvious to Thompson. What was less obvious was how these aspects of growth should be captured mathematically. The solution was described just after the second edition of Thompson's book came out (Richards and Kavanagh, 1943) and set in a firm analytical framework later (Hejnowicz and Romberger, 1984; Silk and Erickson, 1979). To appreciate the solution and its relevance to morphogenesis, we begin with growth in one dimension.

If a line of length L , increases in length by ΔL during a time interval Δt (Fig. 1A), we may ask how a line of twice the length, $2L$, would grow in the same time interval. The answer depends on the mode of growth. If the line grows by addition at one end, the increase would still be ΔL . The growth rate may then be expressed as increment in length per unit time ($\Delta L/\Delta t$), and line length increases linearly with time, $L=kt+A$. This corresponds to situations in which growth occurs by terminal deposition or addition of new material, a process termed 'growth by accretion'. Microtubules grow in this way, by addition of subunits at one end, as do polymers such as cellulose or proteins.

Another possibility is that growth is distributed throughout the line. In this case a line of $2L$ will increase by $2\Delta L$: there is twice as much growing material, equivalent to two lines of L joined end to end, each growing by ΔL . In such a case of evenly 'distributed growth' (also termed 'diffuse growth'), length increment is

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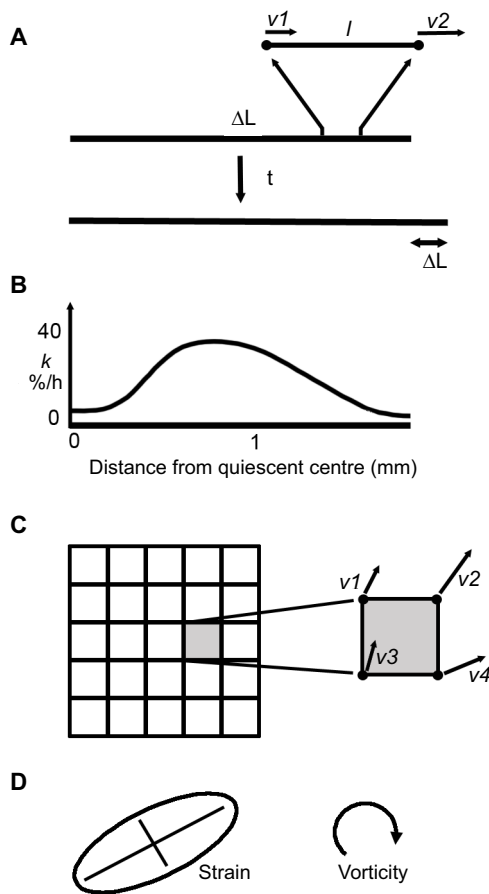


Fig. 1. Describing growth. (A) One-dimensional growth showing growth of line L by ΔL , and line segment l with velocities (v_1 and v_2) of points at each end of the segment. (B) Relative growth rate (k) in length as a function of distance from a slow-growing region at the root tip, termed the quiescent centre [data taken from Beemster and Baskin (1998, 2000)]. (C) Variation in velocities (v_1 – v_4) for the vertices of a small square region (grey) embedded in a tissue. (D) Two-dimensional growth tensor with the strain part shown on left as an ellipse with orthogonal magnitudes. The rotational component (vorticity) is indicated with a curved arrow on the right.

proportional to line length, and the growth rate may be expressed as the percentage increase per unit time ($\Delta L/L \Delta t$) or relative growth rate. Length increases exponentially with time, $L = Ae^{kt}$, with the exponent, k , being the relative growth rate. Growing regions of a young filament of the alga *Anabaena* grow in this manner (Fogg, 1944; Wilcox et al., 1973). Unlike growth by accretion, cells do not become added as subunits to the end of the filament. Instead, growth occurs through internal extension of cells, driven by turgor pressure, with occasional cell divisions keeping cell sizes from increasing indefinitely.

For distributed growth we can estimate local growth rates within our line by tracking the displacement of points or landmarks over time. If we follow two points, either side of a line segment, l , we obtain two velocities v_1 and v_2 (Fig. 1A). If these two velocities are equal, the line segment is not growing but simply moving. If $v_2 > v_1$, the line segment is growing, with the relative growth rate, k , equal to the difference in velocities divided by the length of the line segment, $(v_2 - v_1)/l$. Thus, relative growth rate can be estimated from the rate of change in velocity with distance l ; i.e. by taking the spatial gradient in velocities. For distributed growth, local relative growth

rates need not be uniform. If we consider the main axis of a root as a growing line, the relative growth rate is highest just behind the root tip (Fig. 1B).

If we extend these notions to think about the growth of a two dimensional surface or sheet, growth by accretion corresponds to material being added to an edge instead of an end. Shells, a favourite subject of Thompson's, grow in this way, with calcium carbonate being added to a growing edge (Liew et al., 2014; Meinhardt et al., 2009). Brick walls are also constructed by accretion, with the top of the growing wall providing an edge (one brick wide) to which new bricks are continually added. Growth of a plant tissue sheet, however, does not proceed in this way. The lack of cell rearrangement means that cells cannot be moved from one place to another to be added, brick-like, to a growing edge. Plant growth is distributed and, as with the *Anabaena* filament, growth needs to be described in terms of relative growth rates. Even a pollen tube tip grows in this manner if we look closely enough. There is no growing edge, but a curved surface with regions enlarging at particular relative growth rates. The classical notion of 'tip growth' (Rojas et al., 2011) is therefore equivalent to highly localised distributed growth.

As with growth in one dimension, we can determine relative growth rates for distributed growth in two dimensions by taking the spatial gradient of velocities (Hejnowicz and Romberger, 1984; Richards and Kavanagh, 1943; Silk and Erickson, 1979). Instead of variation in velocities along a line segment, we consider variation around a small area or region (e.g. the grey square in Fig. 1C). However, when we try to take the spatial gradient of these velocities, we encounter a complication, as velocities may vary in direction as well as in magnitude, and may vary to different extents according to whether we move along the horizontal or vertical axis. Thus, unlike the gradient of velocities in one dimension, which can be represented with a single number (relative growth rate), expressing the gradient of velocities in two dimensions requires a different mathematical object, termed a 'tensor' (Hejnowicz and Romberger, 1984; Silk and Erickson, 1979).

A two-dimensional growth tensor can be visualised with an ellipse containing two orthogonal lines (Fig. 1D). The magnitudes of the lines give the relative growth rates along two different axes. In the case of isotropic growth (equal growth in all directions), the two lines have the same length, whereas for anisotropic growth one of the lines is longer. For anisotropic growth, the tensor also has an angle, or principal orientation of growth. Unlike a vector, a tensor has no arrowhead. This is because regional growth does not occur towards or away from any external point. Instead, regional growth is relational, and refers to how material is displaced outwards for that region (or inwards if the material is shrinking). In this respect, growth is similar to stress, which can also be represented with a tensor. Stress refers to how a region is being pulled apart (under tension) or pushed together (under compression) but does not occur towards or away from an external point. In other words, both growth and stress have axiality (orientation) but lack polarity (head and tail ends to their axes).

As with distributed growth in one dimension, relative growth rates need not be uniform across a two-dimensional surface. In such cases, a single growth tensor is insufficient to express the growth pattern. Instead, we need a field of tensors – a tensor for each location on our surface. In the example shown in Fig. 2Ai, isotropic relative growth rate increases linearly from the bottom of a square towards the top, represented by a field of circular tensors of increasing size (the orientation of crosses within them is random), or a greyscale that reflects the magnitude of relative growth rate

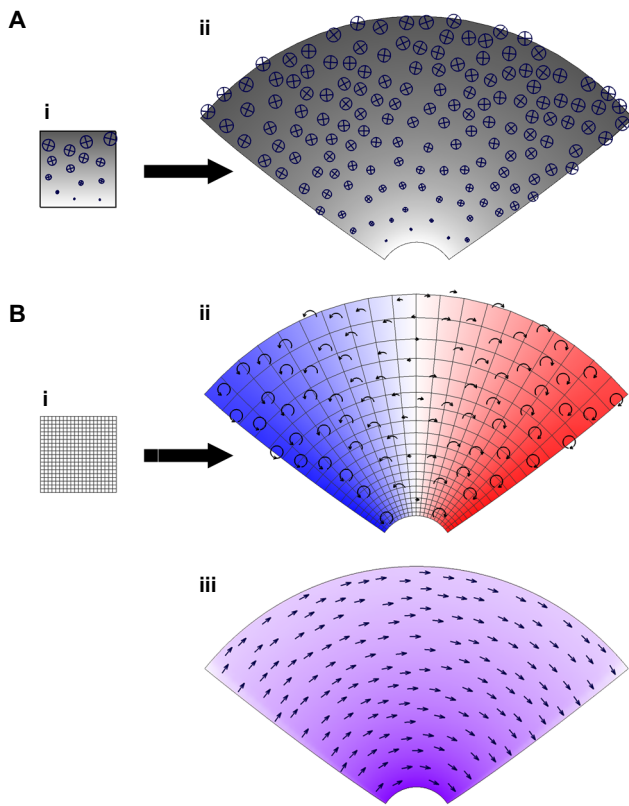


Fig. 2. Specified and resultant tensor fields. (A) Two-dimensional specified tensor field of graded isotropic growth. In the initial square of tissue (i), the specified isotropic growth rate increases linearly from bottom to top, indicated by shading (the darker area indicates highest growth rate) and by strain tensors (circles with crosses inside). The differential growth leads to a fan shape (ii). (B) As in A, but with a grid placed on the initial shape (i) to illustrate the way the tissue curves after growth (ii). Rotation rates for the resultant tensor field in ii are shown with arrows (arc length is proportional to rotation rate) and are colour-coded (blue, anticlockwise; red, clockwise). The rotation rates are not part of the specified tensor field, but only the resultant field. (iii) The gradient of the vorticity gives the local bending rate of the tissue (magnitude is colour-coded purple, direction of the gradient is shown with arrow, paler colour at the side edges is caused by numerical artefact). Simulations were performed using *GFtbox* software (Kennaway et al., 2011).

(darker=higher relative growth rate). As the surface grows, regions rotate relative to each other and the initially straight lines of an initial grid (Fig. 2Bi) become curved (Fig. 2Bii). Such rotations are described with the further component of the growth tensor: the rate at which a region rotates, termed vorticity (Hejnowicz and Romberger, 1984; Silk and Erickson, 1979), indicated by a curved arrow (Fig. 1D) and colour coding (Fig. 2Bii, red clockwise, blue anticlockwise). The gradient of the vorticity gives the local rate of tissue bending (Fig. 2Biii).

Growth of a two-dimensional surface may therefore be described with a field of tensors, each of which can be represented with four quantities: two orthogonal growth rates, the principal orientation of growth and rotation rate (vorticity). The first three quantities form the strain part of the growth tensor, whereas the fourth is the vorticity part (Fig. 1D) (Hejnowicz and Romberger, 1984). Similar considerations apply to the growth of a three-dimensional volume, except that each growth tensor involves nine quantities: three orthogonal growth rates, three angles and three rotation rates, with the first six forming the strain and the last three the vorticity parts.

A growth tensor field describes growth at a given time point, but the field may change over time. A tissue may initially exhibit one pattern of growth, and then change to another. Thus, a ‘dynamic growth tensor field’ is needed to provide a complete continuous description of growth and shape change. The key challenge of morphogenesis is to understand how such fields are generated.

Genes and growth

‘The spiral, or rather helical, geodesic is particularly common in cylindrical structures, and is beautifully shewn for instance in the spiral coil which stiffens the tracheal tubes of an insect, or the so-called tracheides of a woody stem’ (p. 742, Thompson, 1942).

Given Thompson’s interest in helical forms, he would have doubtlessly been fascinated by the structure of DNA. However, Thompson died five years before the structure was published. As well as presenting an intriguing shape, the structure of DNA would also have provided Thompson with a more concrete understanding of what genes are and how they act. DNA is essentially an inert informational molecule that only exerts its effects through interactions with proteins, RNA and other constituents of the cell. Mutations in DNA can modulate these physico-chemical interactions, and through the accumulation of such modulations, the diverse forms of organisms have evolved.

How can discrete mutations in DNA be reconciled with the continuous integrated forms of organisms? In the 1942 edition of his book, Thompson wrote: ‘With the “characters” of Mendelian genetics there is no fault to be found; tall and short, rough and smooth, plain or coloured are opposite tendencies or contrasting qualities, in plain logical contradistinction. But when the morphologist compares one animal with another, point by point or character by character, these are too often the mere outcome of artificial dissection and analysis. Rather is the living body one integral and indivisible whole, in which we cannot find, when we come to look for it, any strict dividing line even between the head and the body, the muscle and the tendon, the sinew and the bone’ (pp. 1036–1037, Thompson, 1942). The contrast between the discrete nature of genes and the continuity of the organism, referred to by Thompson, can be reconciled through two types of coordinating process: chemical and mechanical. ‘Chemical’ here refers to molecular processes described through notions such as concentration, diffusion, reaction rates and flux. ‘Mechanical’ refers to processes described through notions such as stress (compressive or tensile forces per unit area), strain and material properties such as stiffness. For the past few decades, much of developmental biology has focused on the chemical, the mechanisms by which patterns of gene activity are coordinated and refined. It is only more recently that attention has turned to the mechanical, and that is where Thompson’s ideas have come to the fore again; in particular, his ideas about how changes in form may be understood through transformations.

Transformations

‘We are dealing in this chapter with the forms of related organisms, in order to show that the differences between them are as a general rule simple and symmetrical, and just such as might have been brought about by a slight and simple change in the system of forces to which the living and growing organism was exposed’ (p. 1037, Thompson, 1942).

In perhaps his most famous chapter, on the theory of transformations, Thompson envisaged how global deformations, equivalent to the way a fossil might be deformed by the compressive

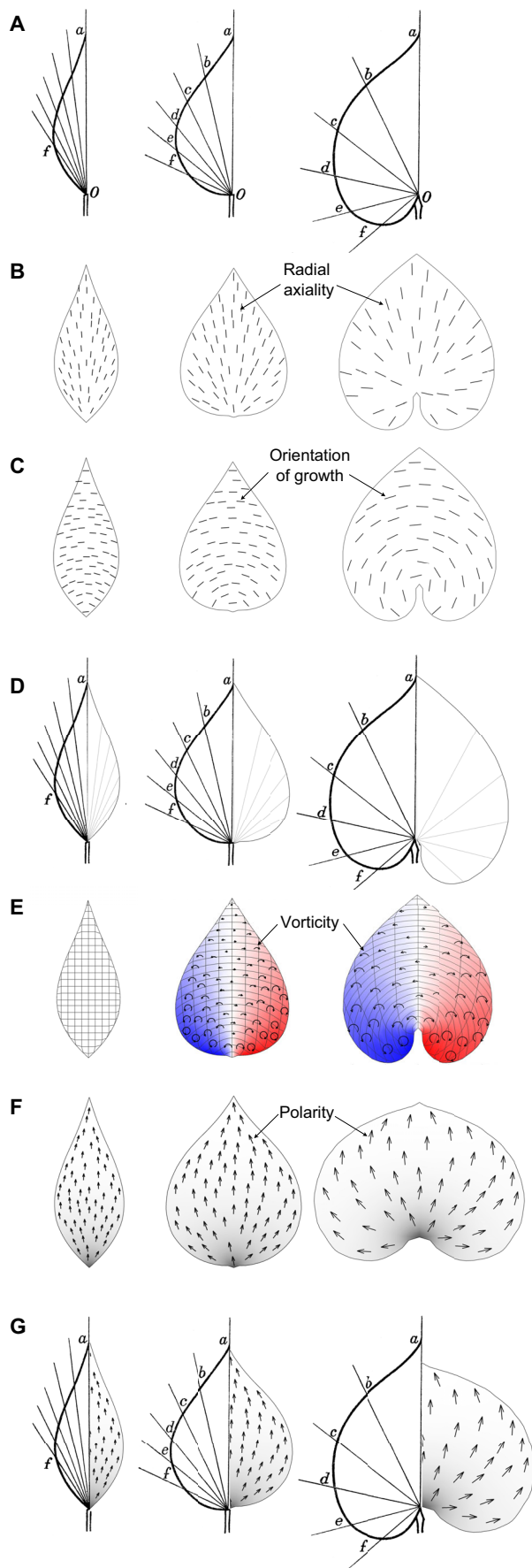


Fig. 3. Generating leaf shape changes. (A) Three leaf shapes from Thompson's book (left, lanceolate; middle, ovate; right, cordiform). Radiating lines labelled *a-f* indicate how the different shapes may be related through tangential growth. (B) Left image: initial shape of Thompson's lanceolate leaf with radiating axially pattern of short lines. Middle and right images: the shapes generated from the lanceolate shape by tangential growth (perpendicular to the axially). (C) Same as B but with specified growth tensors shown as lines perpendicular to axially. (D) Superposition of Thompson's shapes (left hand part of image) on those generated by growth according to the growth tensors shown in C (right hand part of image), illustrating the close match. (E) Same as C but with a square grid superimposed on the initial lanceolate shape (left), showing the grid with rotation rates (curved arrows, and red and blue) and the resultant shapes (ovate and cordiform). (F) A polarity field (arrows) on the initial shape (left) can be generated by producing a diffusible factor at the base, and letting it diffuse and decay (concentration indicated using greyscale). The resulting shapes generated by growth perpendicular to the axially component of the polarity are shown (middle, right). (G) Superposition of Thompson's shapes on F (note the discrepancy of the cordiform shape on the right). The simulation outputs shown in B-G were generated using *GFTbox* software (Kennaway et al., 2011).

and shearing forces of rock, could be used to relate forms of different species (Abzhanov, 2017). He chose relatively flat forms, like those of fishes or leaves, so he could illustrate these deformations in two dimensions. An example is shown in Fig. 3A where he analyses three distinct leaf shapes – lanceolate, ovate and cordiform – that are found in different species. Thompson pointed out that the different forms could be generated quite simply through growth oriented tangential to a coordinate system radiating out from the base of the leaf lamina. Such growth would displace the radial lines evenly and generate the observed leaf outlines.

To gain a clearer understanding of how Thompson's idea can be related to genetics, we need to distinguish between two types of growth: specified and resultant. Specified growth is how a small region of tissue would grow in isolation, disconnected from the rest of the tissue. Resultant growth is the way a small region grows when mechanically connected to the rest of the tissue (Bassel et al., 2014; Kennaway et al., 2011). Thus, specified growth refers to the active or intrinsic growth properties of a region (how the region would 'like' to grow), whereas resultant growth also includes the passive effects or constraints of neighbouring regions (how the region actually grows). These passive effects reflect mechanical forces operating between regions (e.g. cells), and thus provide a mechanical mechanism for integrating growth, ensuring that it operates smoothly or continuously over the tissue. Without mechanical connectivity, the tissue might tear itself apart and exhibit discontinuities. The degree of mechanical connectivity can vary. For example, the epidermal layer of the developing leaf has tightly connected cells (with the exception of stomatal pores), whereas subepidermal layers exhibit intercellular spaces, indicating that its cells are less strongly connected.

If all regions of a tissue have the same specified growth rates and orientations, the tissue simply enlarges or stretches, and resultant and specified growth are equal. However, if specified growth varies across the tissue, potential conflicts may arise between connected regions trying to grow in different ways (Coen and Rebocho, 2016). In this case, specified and resultant growth tensors may differ, as regions are unable to attain their specified dimensions. In addition, the resultant tensors may include vorticity components as regions rotate relative to each other to reduce or resolve potential conflicts and stresses. Unlike resultant growth, there are no rotational (vorticity) components in the specified growth tensor, as we generally assume there is no intrinsic force driving the rotation of regions.

We can illustrate specified and resultant growth by returning to Thompson's leaf example and attempting to model the transformations he described. We begin with a sheet, or canvas, of connected tissue in the shape of his leaf on the left (Fig. 3B left). In accordance with Thompson's idea, we assume a radial pattern of orientations centred on the base of the leaf lamina, illustrated by the axiality field (short lines) on the canvas. Suppose each region of our canvas has specified growth perpendicular to its local axiality (tangential to the radial system, in Thompson's terms). The specified growth tensor field can then be represented with a series of ellipses with their longer axis oriented perpendicular to the axiality (Fig. 3C, left). In this case, the ellipses collapse to lines as growth along the minor axis of the ellipses is zero. Applying this specified growth pattern causes the canvas to go through a series of shape transformations (Fig. 3B-D) that are almost identical to those Thompson drew.

The resultant growth tensors for the canvas are similar to the specified growth tensors, except that they also have a rotational component, evident from the way the lines of an initially square grid become curved (Fig. 3E). If regions did not rotate in this manner, stresses would arise as nearby regions try to grow in different orientations. Rotations may therefore be seen as a way of resolving potential conflicts in growth. In this case, potential conflicts are fully resolved and no stresses are generated.

This example raises a key question – how might a radial axiality field be generated in a biological tissue and maintained as the tissue deforms? One possibility is that the axiality field has a mechanical origin, arising from a radial pattern of stresses. In this case, the stresses cannot be generated within the tissue through differential planar growth, as potential growth conflicts are fully resolved through rotations. There would therefore have to be an externally derived stress pattern and this would need to be kept radial, even though the shape of the leaf is not radially symmetrical about the centre of the field. It is difficult to see how such a stress pattern could be generated and maintained during growth. For example, an externally derived stress generated at the base of the lamina could radiate out near the base, but would then be constrained to follow the outline of the leaf.

Alternatively, the axiality field could have a chemical basis. Suppose a particular type of molecule is continuously generated at the base of the leaf lamina and diffuses and decays through the lamina (Fig. 3F, left, shaded area). The local gradient in concentration of this molecule would then provide a set of directions, or a polarity field, that radiates out from the leaf base (Fig. 3F, left). Here, polarity is represented with a vector of unit length, and thus exhibits both axiality (orientation) and sense (arrowhead). If we now orient specified growth perpendicular to the axiality component of the polarity field, we end up with a series of shapes (Fig. 3F, middle and right). However, the match with Thompson's shapes is not as good as for the perfect radial field, particularly for the cordiform shape on the right (compare Fig. 3G with Fig. 3D). The discrepancy arises because the polarity field tends to follow the leaf outline and converge in more distal regions, rather than being maintained as perfectly radial. Thus, when we come to put the geometrical transformations that Thompson envisaged into practice, with either chemical or mechanical mechanisms for orienting growth, we find it is not as straightforward as might have been envisioned.

Developmental transformations

'To turn one circle (or sphere) into two circles (or spheres) would be, from the point of view of the mathematician, an extraordinarily difficult transformation; but physically speaking, its achievement may be extremely simple' (p. 1049, Thompson, 1942).

Thompson illustrates this idea of the ease of physical transformations with a gourd – a fruit of the cucumber family. If a rag is tied around the middle of a young gourd, it grows to form two connected globes, a non-trivial mathematical transformation. Thompson's realisation that complex geometrical transformations may be achieved simply through growth was insightful. What Thompson did not perhaps appreciate so clearly is that the converse also applies: simple geometric transformations may be hard to achieve through biological growth. The example of the leaf given above is a case in point. It is simple to imagine an externally imposed radial coordinate system, but can be harder to establish one through internal mechanisms. Similarly, simple geometric operations, such as transformation of a square into a trapezoid, can be difficult to achieve biologically because of the rotations and curving generated through differential growth. Thompson's oversight may derive from his lack of knowledge of how internal process pattern and orient growth. His analogies drew on externally imposed constraints or forces – the compressive action of rock on a fossil, a rag tied round a gourd, a glassblower guiding molten glass – rather than internally derived ones.

There is a further problem in relating Thompson's insights on geometric transformations to living systems. As a tribute to D'Arcy Thompson completing 60 years as a professor, a collection of essays was published at the end of the Second World War. Peter Medawar discussed Thompson's 'Method of Transformations' in his essay: 'There can be no doubt, as Richards and Riley (1937) foresaw, that its true field of application lies in development, not evolution; in the process of transforming, not in the fait accompli' (Medawar, 1945). Thompson applied his method to comparing forms of different species, but as Medawar and others pointed out, evolution does not involve a physical transformation of one mature form into another; each form arises separately through the process of development.

For the leaf, Thompson explained why he chose to apply his method to evolutionary rather than developmental shape changes: 'These successive changes may to some extent, and in appropriate cases, be traced as the individual leaf grows to maturity; but as a much more general rule, the balance of forces, the ratio between radial and tangential velocities of growth, remains so nicely and constantly balanced that the leaf increases in size without conspicuous modification of form' (p. 1042, Thompson, 1942). Leaf shape hardly changed during development, so it was with comparisons between species that his approach proved most illuminating. Had Thompson looked at earlier stages of leaf development, he would have seen that the leaf does undergo marked transformations in shape during its morphogenesis. Moreover, had he been able to follow the landmarks such as cell vertices, he would have seen coordinate transformations just as dramatic as those he showed through evolutionary comparisons (Kuchen et al., 2012; Solly et al., 2017). Even so, Thompson was not worried by the fact that the transformations he studied did not correspond to physical transformations. He was more concerned with general geometrical principles. These principles led him to conclude that there must be coordinate patterns of growth underlying the generation of forms. Although the value of this insight should not be underestimated, defining what the growth patterns were, how they were established and how they led to the observed transformations remained obscure. To address these problems, we need to understand how genes influence specified growth.

The cellular basis of specified growth

'The cell, which Goodsir spoke of as a centre of force, is in reality a sphere of action of certain more or less localised forces' (p. 341, Thompson, 1942).

The cell is a natural starting point for considering how the specified growth tensor of a tissue region is established and genetically regulated. This is because the cell is not only, as Thompson pointed out, a sphere of action for physical forces; it is also a sphere of gene action, as each cell has its own DNA copies expressed in a particular way.

Plant cells grow through a balance between two forces: expansive turgor pressure and restraining forces of the cell wall. If the restraining forces are insufficient to completely counteract turgor, the cell wall gradually extends (Cosgrove, 2016). Genes may influence the specified growth rates of a cell (i.e. how that cell would grow in mechanical isolation from other cells) by modifying osmotic pressure or cell wall properties. Osmotic pressure acts equally in all directions (isotropic) whereas the cell wall may be anisotropic and yield more in some directions compared with others. To understand coordinated patterns of anisotropic growth, of the kind Thompson envisaged with his leaf transformations, we must therefore turn to how the material properties of the cell wall are established.

The anisotropic yielding properties of plant cell walls depend on the orientation and crosslinking (e.g. via pectins or xyloglucans) of cellulose fibres (microfibrils) (Cosgrove, 2005). If cellulose fibres are preferentially oriented in one direction, the cell will yield or grow preferentially in the orthogonal direction, as this is the orientation of least stiffness (Baskin, 2005). The orientation of cellulose fibres in turn depends on the orientation of microtubules, which guide cellulose synthases in the plasma membrane (Chan et al., 2010; Gutierrez et al., 2009; Paredes et al., 2006). Microtubules typically display aligned patterns within a cell, though these alignments may change over time (Chan et al., 2010). Individual microtubules grow by accretion – addition of tubulin at the plus end – so how does the growth orientation of one microtubule become aligned with another to give a coordinated pattern in the cell?

One possibility is that alignments arise through redirection of microtubules following encounters. For example, when the plus end of a microtubule encounters an obstructing microtubule, the plus end may change its orientation of growth to follow that of the obstructing microtubule, a process termed zippering (Dixit and Cyr, 2004). Computer simulations show that zippering can promote alignment formation under certain conditions (Allard et al., 2010). Alignments may also arise through ‘differential survival’ following microtubule encounters (Tindemans et al., 2010). When a growing plus-end encounters an obstructing microtubule, the plus end may undergo rapid depolymerisation, a process termed catastrophe. Repeated catastrophes in a population of microtubules can cause alignments to emerge (Tindemans et al., 2010). This is because a slight excess of microtubules in one orientation gives a survival advantage to microtubules growing in the same orientation (they are less likely to undergo catastrophe), which further reinforces the alignment. Both redirection and differential survival provide potential mechanisms for generating microtubule alignments. The issue remains of how such alignments are coordinated between cells and across tissues.

Coordinating specified growth

‘Every growing organism, and every part of such a growing organism, has its own specific rate of growth, referred to this or that particular direction; and it is by the ratio between these rates in different directions that we must account for the external forms of all save certain very minute organisms’ (p. 82, Thompson, 1942).

Thompson’s eloquent statement raises two questions: (1) how are growth rates coordinated; and (2) how are the orientations of growth

coordinated? Let’s return to Thompson’s leaf but now consider how its shape might be generated through development rather than evolution. We can represent the shape of a leaf primordium with a simplified form with a square grid inscribed on it, shown on the left of Fig. 4A. The problem is how this initial shape might be transformed into the lanceolate shape on the right through genes that modulate growth.

Given that our target lanceolate leaf shape bulges out in the middle and tapers at the base and tip, we might postulate a growth-promoting transcription factor to be expressed in the middle region of our primordium (Fig. 4B, left). If this factor promotes specified isotropic growth (e.g. uniform weakening of cell walls), the resultant growth gives the shape shown on the right of Fig. 4B. However, this leaf is rounded rather than elongated. Thus, it is not straightforward using specified isotropic growth alone to generate the target lanceolate shape.

Perhaps the shape can be more readily generated through coordinated patterns of specified anisotropic growth. Expression levels of a transcription factor alone are insufficient for this purpose, as these correspond to scalar values that carry no orientation information directly (Lawrence et al., 2007). We therefore need a mechanism for establishing a pattern of coordinated orientations. Such a mechanism could then influence growth by biasing the alignments of microtubules in favour of particular orientations. As we discuss below, two possible mechanisms for coordinating orientations of specified anisotropic growth have been proposed: stress axially and polarity.

Coordinating specified growth orientations by stress axially

Stress anisotropy is attractive as a mechanism for orienting specified growth because, like growth, stress is a tensor. Possible source of stresses are external constraints on the growing tissue, such as the restraining forces exerted by surrounding leaves, or the packing of a leaf within a bud (Couturier et al., 2011). However, *Arabidopsis* leaves are able to form their shape in a growth chamber, where much of the growing lamina is free from external constraints, suggesting that externally derived stresses are not essential for coordinating growth.

Another possible source of stresses is differential growth within the tissue. For example, if the lower (proximal) half of our primordium has a lower specified growth rate than the upper (distal) half (Fig. 4Ci), stresses are generated (Fig. 4Cii). Anisotropic stresses arise because of mechanical connectivity: growth of the upper half is restricted by the lower half, whereas growth of the lower half is stretched (put under extra tension) by the upper half. These residual stresses reflect growth conflicts that cannot be fully resolved by rotations.

In principle, the local residual stresses generated by such conflicts might be used to orient specified growth (Hervieux et al., 2016). Consider the lower part of the primordium in Fig. 4Cii where tension is maximal horizontally. If cells in this region reinforce themselves along the direction of maximal tension, so as to resist the tensile force (the common assumption for stress-based models), they would grow faster in the vertical direction if grown in isolation (because they are now less stiff in this direction). Within the tissue context, these cells experience a lower stress vertically than horizontally (maximal tensile stress is horizontal). To generate a higher rate of vertical relative to horizontal resultant growth, the cells would therefore have to be so stiff in the horizontal direction that vertical growth is favoured, despite the lower tensile stress in this direction. However, if cell walls are reinforced in this manner, by incorporation of additional

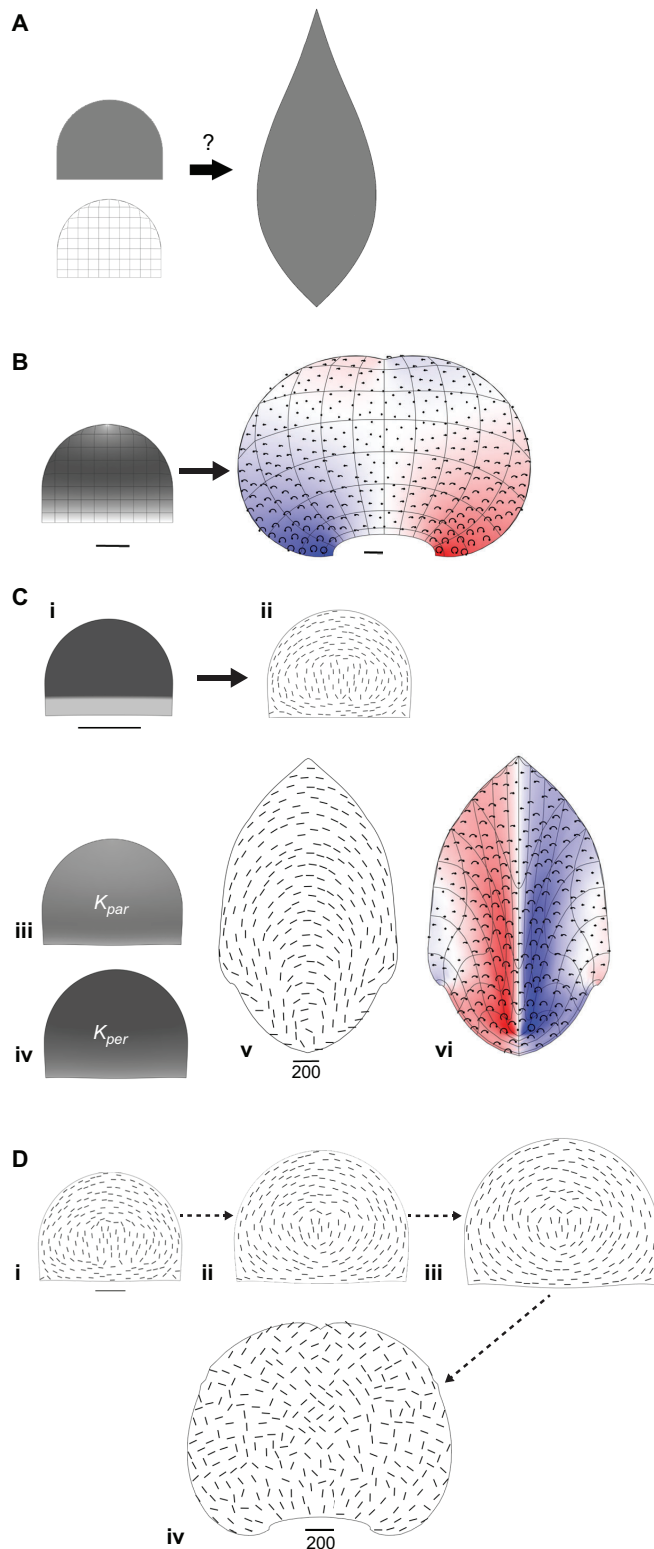


Fig. 4. Generating Thompson's leaf shape through stress-based axially. (A) Initial primordium shape (left) with or without a square grid inscribed, and the target lanceolate shape to be generated by growth (right). (B) Isotropic specified growth driven by an initial growth-promoting factor with lower levels at the base and tip (left). The middle region of the primordium swells to generate a fat leaf, with deformation of grid and rotation rates shown (right). The scale bar indicates the relative sizes of the tissue before and after growth. (C) An initial pattern of higher isotropic growth in the upper part of the primordium (dark grey, i) generates residual stresses (ii), with axially (short lines) aligned with direction of local maximal tension. Specified growth may then be expressed as differential rates parallel (K_{par} , iii) and perpendicular (K_{per} , iv) to the orientation of this axially (higher rates shown in dark grey). If the pattern of stress-based axially is fixed to the tissue and deforms with it, a lanceolate leaf can be generated (right), shown with the axially pattern (v) and rotation rates within the deformed grid (vi). i-iv are at the same scale. By comparison, v and vi are much larger, indicated by the scale bar which is 200 times longer. (D) Same growth rules as in C but updating axially pattern according to the stresses generated. After the initial pattern of stresses is established (i), growth is specified in relation to the axially (as in Ciii and Civ). This generates a new pattern of stresses (ii) that updates the axially pattern. Specified growth oriented by this updated pattern then leads to another pattern of stresses (iii). Continuing in this manner leads to the shape shown in iv. The size of iv is much larger than i-iii, indicated by the 200 times greater length of the scale bar. The simulation outputs shown in B-D were generated using *GfTbox* software (Kennaway et al., 2011).

To circumvent such difficulties, it has been proposed that residual stresses are spatially averaged over the organ to give an overall orientation of stress (Hervieux et al., 2016). However, it is unclear what mechanism could generate such spatial averaging and how a cell would distinguish average stress from local stress (as both stresses have the same physical nature). Moreover, reinforcing cell walls in the orientation of average stress reduces the stress in that direction (by increasing cross-sectional area of microfibrils), promoting a switch in average stress to the perpendicular orientation.

Another possibility is that the axially pattern derived from stresses becomes fixed to the tissue at an early stage, avoiding the problem of growth feeding back to modify stress patterns. For example, suppose the local direction of stresses in Fig. 4Cii is used to define an axially pattern that becomes fixed to the tissue and grows with it. We may define two rates of specified growth: specified growth rate parallel to the axially (K_{par}) and specified growth rate perpendicular to the axially (K_{per}). These two growth rates correspond to the magnitudes in the strain part of the specified growth tensor (Fig. 1D). These magnitudes could be under the control of transcription factors that influence the extent to which axially biases anisotropies in the cell walls. For example, if the transcription factors with the distributions shown in Fig. 4Ciii,iv control K_{par} and K_{per} respectively, the resultant growth leads to a lanceolate leaf shape (Fig. 4Cv,vi; an equivalent result could be obtained by transcription factors controlling specified anisotropy and areal growth rate instead of K_{par} and K_{per} separately). To illustrate how this mechanism may depend on fixing the axially pattern, suppose we allow stresses generated during growth to feed back and update the axially pattern. After a growth step, the pattern of specified growth leads to a change in the stress pattern (compare Fig. 4Dii with Fig. 4Di). If the axially pattern is now updated according to this new pattern of stresses and used to orient growth, we obtain yet another pattern of stresses (Fig. 4Diii). Continuing in this manner we end up with the shape shown in Fig. 4Div. Although these simulations make simplifying assumptions, they illustrate the point that, unless the axially pattern is fixed from an early stage, it may be difficult to create a stable oriented pattern of specified growth through a stress-based mechanism, at least for planar tissues, because resultant growth continually feeds back to modify the residual stresses and thus the pattern of growth.

cellulose microfibrils, the stress they experience in the horizontal orientation is reduced. This is because stress is force divided by the cross-sectional area of the load-bearing material (cellulose microfibrils in this case), and with more microfibrils, the cross-sectional area increases, reducing stress. Thus, the principal direction of stress may now change from horizontal to vertical, which would in turn switch the direction of cell stiffening and thus growth orientation.

Coordinating specified growth orientations by polarity

Another proposed mechanism for orienting specified anisotropic growth involves cell polarity. Cell polarity (molecular asymmetry between different ends of a cell) has been revealed through the localisation of proteins such as the PIN auxin transporter (Friml et al., 2003). Cell polarities may be coordinated between cells to give a tissue cell polarity pattern (Kuchen et al., 2012; Sauret-Güeto et al., 2013). Several chemical mechanisms have been proposed to account for such coordinated tissue cell polarity patterns, including localisation of PINs up gradients of intracellular auxin, localisation in the direction of auxin flux, and cell-cell coupling according to extracellular auxin concentrations (Abley et al., 2013, 2016; Feugier et al., 2005; Jönsson et al., 2006; Rolland-Lagan and Prusinkiewicz, 2005; Stoma et al., 2008). In these models, auxin sources or sinks can provide regions, or organisers, that anchor cell polarities to gene expression domains. For example, in the leaf primordium shown in Fig. 5Ai, polarity points away from an organiser at the base of the leaf (the plus organiser). In this example, polarity is modelled by taking the gradient of a diffusible molecule generated at the primordium base (source), as a proxy for the tissue cell polarity mechanism.

As with the stress-based axiality pattern described above, given a pattern of tissue cell polarity we may define two rates of specified growth: specified growth rate parallel to the polarity (K_{par}) and specified growth rate perpendicular to the polarity (K_{per}). If transcription factors with the distributions shown in Fig. 5Aii,iii control K_{par} and K_{per} respectively, the resultant growth generates a good match to our target lanceolate shape (Fig. 5Aiv,v). A similar result is obtained if we fix polarity to the tissue from an early stage rather than letting it continually adjust through diffusion (Fig. 5Avi). Thus, tissue cell polarity provides a robust mechanism for orienting specified growth.

In the example shown in Fig. 5A, the model is constrained only by how well its output matches the target outline. There are many patterns of specified growth (distributions of K_{par} and K_{per}) that can generate such an outline. When experimentally analysing leaf growth, these possibilities are further constrained by having to account for the observed distribution of growth within the leaf (observed growth tensor field), estimated by tracking cell vertices or through clonal analysis (Eldridge et al., 2016; Kuchen et al., 2012; Sauret-Güeto et al., 2013).

With a chemically based polarity model (Fig. 5A), the shape generated depends on an interplay between chemical and mechanical coordinating mechanisms. Chemical mechanisms determine the coordinated pattern of specified growth; mechanical mechanisms further coordinate tissue deformation through tissue connectivity (e.g. the stresses cells exert on each other), yielding resultant growth. The chemical and mechanical mechanisms interact because as the tissue becomes deformed through resultant growth, the shape change may influence the distribution of chemical factors, which in turn influence specified growth. This feedback allows geometrically complex shape transformations to be generated through relatively simple growth rules, a feature of growth emphasised by Thompson.

A further option might be to use a mechanical mechanism (stresses) to orient polarity (Bringmann and Bergmann, 2017; Heisler et al., 2010). Stress alone cannot orient polarity, as stress has no arrowhead, but if we take the gradient in the magnitude of stress (rather than using the orientation of stress), we obtain a vector that could be used to orient polarity (Heisler et al., 2010). Returning to our leaf primordium, suppose we have a higher specified isotropic growth at the distal tip (arrowed in Fig. 5Bi). After time, this

generates a gradient in the magnitude of residual stresses that can be used to define a polarity field (Fig. 5Bii). We may then use this polarity field in the manner of a chemical polarity field to orient specified growth parallel (Fig. 5Biii) or perpendicular (Fig. 5Biv) to the polarity field. If we fix the polarity field to the tissue, such a model can generate a lanceolate leaf (Fig. 5Bv,vi). However, if we allow the polarity field to be continually updated by the gradient of residual stresses generated through growth (Fig. 5Ci-iii), we obtain a different shape (Fig. 5Civ). Thus, using stress gradients to generate a polarity field is less straightforward than using a chemical mechanism because the stresses generated may continually feed back to modify the polarity field and specified growth orientations.

However, using chemically based polarity rather than stresses to orient specified growth is less economical. Stresses are generated for free by differential specified growth or tissue geometry, whereas we need to postulate an additional chemical mechanism to generate and coordinate cell polarity. Moreover, polarity has an arrowhead, which is superfluous in the specification of growth orientation, whereas stresses carry only the axiality required. However, economy can bring costs in terms of flexibility. Using stresses as the primary mechanism to orient anisotropic specified growth may put constraints on achievable growth patterns because specified growth orientations may be continually subject to the stresses they generate. Using chemically based cell polarity does not suffer from these problems as orientations can be specified independently of the stresses generated. Thus, whereas stresses play a key role in driving growth and may also feed back to influence cell wall mechanical properties (e.g. via residual stresses), they may not be sufficient alone to account for the coordinated orientations of specified anisotropic growth involved in the generation of planar organ shapes.

Evolution

‘Darwin’s well-known disquisition on the ocellar pattern of the feathers of the Argus pheasant, as a result of sexual selection, will occur to the reader’s mind, in striking contrast to this or any other direct physical explanation’ (p. 664, Thompson, 1942).

In several chapters of his book, Thompson contrasts explanations based on physico-chemical mechanisms with those based on natural selection. To him, the spots on a pheasant’s feather could be explained by physico-chemical mechanisms like diffusion, rather than by speculative theories of sexual selection. From today’s perspective, these two types of explanation – physico-chemical versus selection – may be seen as complementary rather than contradictory. The evolutionary biologist Ernst Mayr classified biological explanations into two types: proximate and ultimate (Mayr, 1961). The developmental (or functional) biologist wants to understand the physico-chemical mechanisms by which phenotypes are generated during the lifetime of an individual – the ‘proximate’ cause. The evolutionary biologist wants to explain the origins of the genotype and why particular phenotypes might have been selected for over multiple generations – the ‘ultimate’ cause. Mayr noted that ‘In the first third of this [20th] century, no one seems to have realised the existence of these two markedly different kinds of causations. As a result, even when dealing with the same phenomenon, the opponents talked past each other’ (Mayr and Provine, 1980). Thompson was no different from others in his time in confounding these types of explanation.

Although distinct, proximate and ultimate causes are connected because it is the variation in the process of development that provides the raw material for evolutionary change. If a shape is

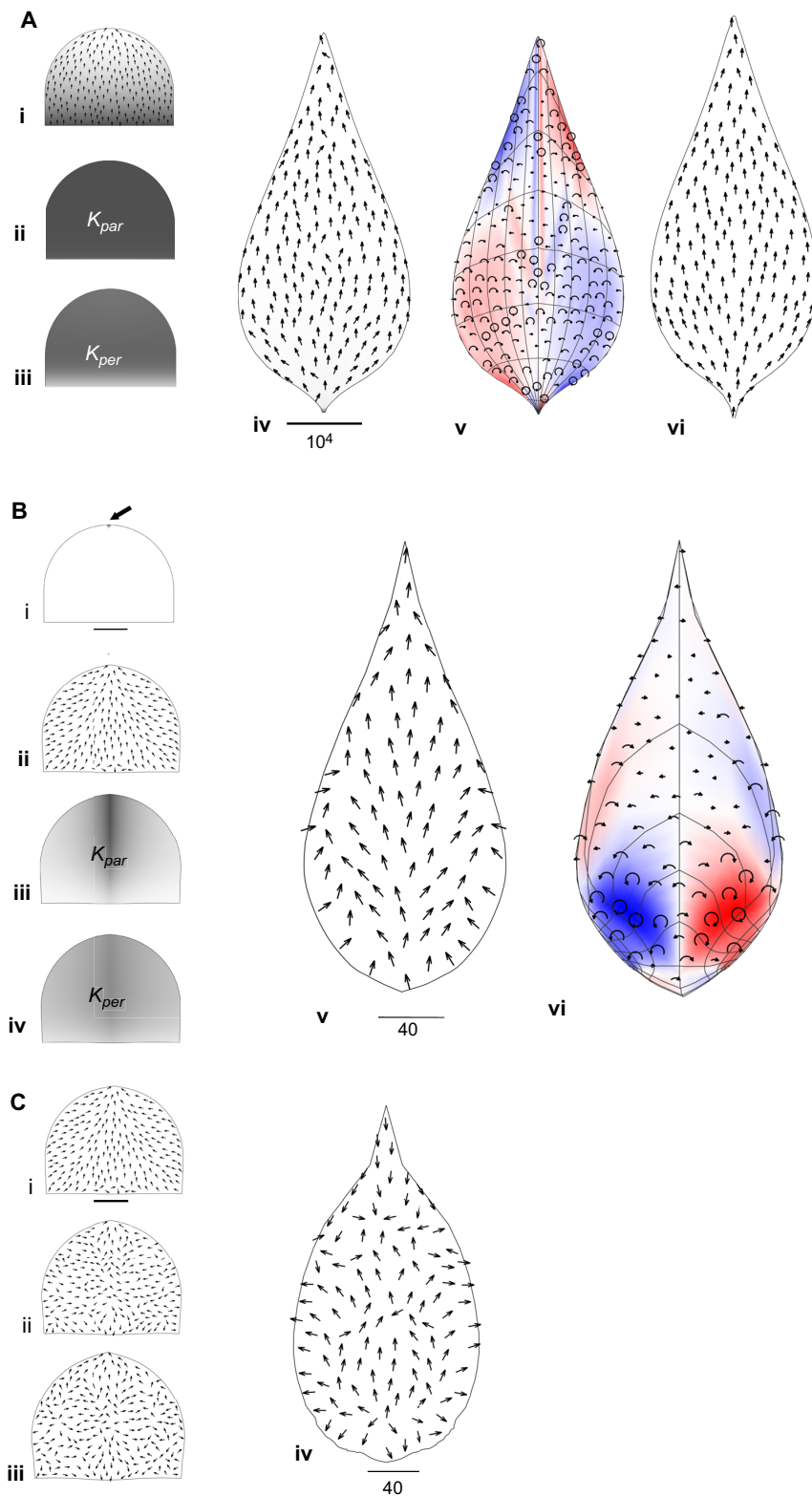


Fig. 5. Generating Thompson's leaf shape through a polarity-based mechanism for orienting growth. (A) Polarity established through a chemical mechanism, by taking the gradient in concentration of a molecule produced at the base with a constant rate of diffusion and decay (i). Growth rates are specified parallel (K_{par} , ii) and perpendicular (K_{per} , iii) to the polarity (with higher rates shown in darker grey). The resulting lanceolate shape is on the right, showing the polarity (iv) or vorticity and grid deformation (v, initial grid as in Fig. 4A). (vi) A similar result is obtained if polarity is fixed to the tissue from an early stage rather than continually diffusing. (B) Polarity established by a stress gradient (mechanical mechanism). Higher specified growth at the tip (arrows in i) leads to a gradient of residual stresses. This gradient can be used to establish a polarity field (ii) with arrows pointing towards regions of greater stress. Specified growth can be defined parallel (K_{par} , iii) and perpendicular (K_{per} , iv) to the polarity field (higher rates shown in darker grey). If the polarity field is fixed to the tissue, a lanceolate leaf shape can be generated, illustrated together with polarity (v) and deformed grid and vorticity (vi, initial grid as in Fig. 4A). (C) Polarity established by a stress gradient with updating. The same growth rules apply as in B, but polarity is updated according to stress gradients generated. After the initial stress gradient is established (i), growth is specified in relation to the polarity (as in Biii and iv). This generates a new pattern of stress gradients (ii) that updates the polarity pattern. Specified growth oriented by this updated pattern then leads to another pattern of stress gradients (iii). Continuing in this manner leads to the shape shown in iv. All initial shapes on left of the figure are at the same scale (shown in Bi and Ci), whereas those on the right are larger, with size relative to initial tissue indicated by the increased length of the scale bar. The simulation outputs shown in A-C were generated using *GFitbox* software (Kennaway et al., 2011).

difficult to generate developmentally, then it may not evolve so readily, even if it confers an adaptive advantage. Conversely, a shape that strikes us as beautifully adapted may arise through simple developmental rules rather than requiring extensive honing through natural selection. A full understanding of leaf shape therefore requires appreciating both developmental and evolutionary processes, and how they interact.

For the three leaf shapes described by Thompson (Fig. 3A), we can ask how easily they might be generated through modulation of gene activity. That is, rather than asking about how we might transform one mature shape into the other, as Thompson did, we can ask about how each shape might be generated from a primordium by modulating development. The shape on the left (lanceolate) can be produced by orienting specified growth with a chemically based

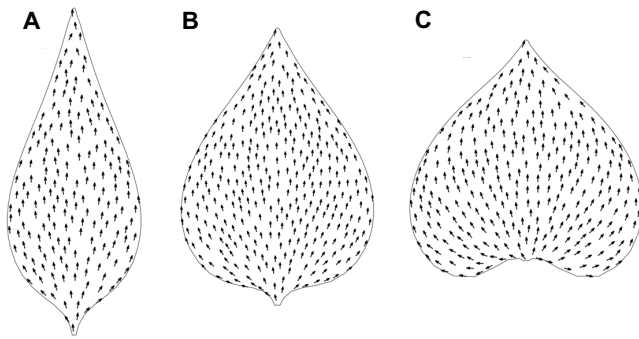


Fig. 6. Generating Thompson's three leaf shapes by modulating developmental growth. (A) A lanceolate shape, generated as in Fig. 5A*vi*. (B) An ovate shape generated as in Fig. 5A*vi* but with a small increment in K_{per} throughout. (C) A cordiform shape generated as in Fig. 5B, but with a further small increment in K_{per} , and additional slight modifications in growth equations to compensate for the fact that the polarity field is not radial. In all three examples, arrows indicate the polarity field in the final shape. The simulation outputs shown in A–C were generated using *GfTbox* software (Kennaway et al., 2011).

polarity mechanism (Fig. 5A, shown again in Fig. 6A). If we now increase the level of the transcription factor that promotes K_{per} , we end up with the ovate shape (Fig. 6B). Further increasing this factor, combined with additional modulations, leads to the cordiform shape (Fig. 6C). Such modelling supports Thompson's insight that different shapes may reflect simple modulations of coordinated growth. However, the coordinate patterns are not those of simple geometry; they arise through genes modulating internal patterns of specified growth.

The above example is largely theoretical and much effort is now going into identifying the relevant genes, how they may be incorporated in growth models, and how they may vary between species to confer different shapes. For example, rather than models based on outlines, those constrained by observed growth or expression patterns may be modulated to generate different shapes (Kuchen et al., 2012; Runions et al., 2017; Rebocho et al., 2017; Green et al., 2010). The role of specific genes is also being explored. For example, the expression pattern of the gene *RCO* controls differences in leaf complexity between *Arabidopsis* and *Cardamine* by repressing growth at specific regions (Vlad et al., 2014; Vuolo et al., 2016). *CUC* genes also play a major role in controlling the serrated outline of leaves, and variation in *CUC* expression between species has been related to the degree of leaf dissection (Blein et al., 2008). *CUC* expression has also been integrated within models for leaf patterning and shape (Bilsborough et al., 2011). Similarly, genes conferring different fruit shapes have been identified (Eldridge et al., 2016; Sinnot, 1922; Tanksley, 2009) and have begun to be incorporated into growth models to explain how diversity in fruit shape may evolve (Eldridge et al., 2016). These studies are beginning to provide us with an understanding of shape development in each species and how underlying gene activity may vary between species. There is also the further challenge of relating these findings to ultimate causes, the population genetic and ecological factors that influence the evolution of shape.

Conclusions

On Growth and Form is a profound book, not only in the ideas and approach presented, but also in identifying problems for the future.

For many years these problems lay dormant. Referring to Thompson's method of transformations, Peter Medawar wrote 'though he expounded it with marvellous lucidity, the method has been put to little further use' (Medawar, 1945). Key advances needed to take the work further came from genetics, a field Thompson largely left aside. Not only have we identified genes that modify growth and form, we have begun to understand how patterns of gene expression are elaborated during development, how cell polarities are coordinated, how stresses may be generated, how mechanical properties of cell walls may be determined, how genes might modulate specified growth, how mechanical connectivity leads to resultant growth and how all of these processes might be modulated during evolution to generate diverse forms. The divisions between genetics, natural selection, and the mechanics of growth and form evident in Thompson's book are being resolved as ideas and findings from different disciplines come together. Even so, we are still ignorant of many of the underlying molecular mechanisms. We have many plausible hypotheses but do not know whether these or others account for how gene activity modifies the cytoskeleton and cell wall, how these modifications lead to specified and resultant growth rates, how polarity is propagated, how stresses interact with cells, or how and why growth patterns are modified during evolution. It is perhaps appropriate to end with the same words that D'Arcy Thompson used near the end of his book 'Our simple, or simplified, illustrations carry us but little way, and only half prepare us for much harder things' (p. 1090, Thompson, 1942).

Competing interests

The authors declare no competing or financial interests.

Funding

The authors' research was supported by grants to E.C. from the UK Biotechnology and Biological Research Council (BB/J004588/1 and BB/F005997/1) and by the European Research Council grant Carnomorph.

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