

FUNDAMENTAL CONCEPTS OF LEAF MORPHOLOGY AND MORPHOGENESIS: A CONTRIBUTION TO THE INTERPRETATION OF MOLECULAR GENETIC MUTANTS

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As an older and more established discipline, plant morphology is an important but relatively underutilized resource for the interpretation of mutants. Plant morphology has the potential to provide the context for molecular genetic investigations, especially those concerned with leaf morphogenesis. This article first reviews the basic organization of vascular plants and the relationship of anatomy to morphology in interpreting gene expression. It demonstrates that these two levels of organization are relatively independent of one another and that anatomy cannot be substituted for morphology in analyzing the effects of genes on leaf development. Morphological principles are then applied to two aspects of leaf development, transectional symmetry and leaf dissection. According to this perspective, so-called organs in plants are simply regional expansions that carry specific cell and tissue types in a particular orientation in space. Rather than considering specific genes as determining particular organ types, it is more likely that gene expression itself is dependent on the morphological, developmental context in which the genes are expressed.

Keywords: histogenesis, interpreting mutants, leaf development, leaf morphology, molecular genetic mutants, morphogenesis, plant morphology.

Introduction

As a scientific discipline, plant morphology is more than 210 yr old, having been originated by Goethe in 1790 (Kaplan, in press). Plant morphology is a comparative discipline concerned with form relationships and the principles they express from the whole plant to the organ level of organization (Kaplan, in press). Since molecular genetics uses deviations in phenotype to deduce gene function, the extensive characterizations and deductions of the morphological principles underlying these deviations (Troll 1937–1943) are key resources to the proper interpretation and understanding of the mutants investigated by the new molecular technology. In short, if molecular genetic studies provide the text, plant morphology provides the context for their interpretation. Many molecular geneticists have not considered this context, and some have even attempted to derive the context from the text. Many recent articles in the molecular genetics of leaf development have disregarded plant morphology as a resource and have instead reinvented it. Some have applied animal-based concepts and terminology where they do not fit. This article attempts to reconnect genetic studies with this morphological tradition to allow a more effective coupling between phenotype and genotype.

Morphological literature may have been disregarded by molecular geneticists because a good part of it is in foreign languages (principally German), but a more basic difficulty is that many contemporary workers are not able to observe a spec-

trum of organismal forms and deduce a common theme from it. It is assumed that once the gene for a given characteristic is identified, then that genetic information supersedes conclusions based on form relationships. Thus, there has been a tendency to lose sight of the connection between the genotype and the phenotype.

The most notable recent studies of molecular genetics have established the context before they evaluated their mutants. For example, the studies of Elliot Meyerowitz and coworkers on floral homeotic mutants in *Arabidopsis* were successful because they had extensively surveyed the past literature of comparative floral morphology (Meyerowitz et al. 1989) and, therefore, knew the basic homologies between the floral whorls whose determination they were studying. Given that Meyerowitz's work is held by many to be a model study in the molecular genetics of plants, it is remarkable how few workers seem to have grasped the morphological context for its success.

In this article, I deal with two contemporary problems in leaf morphogenesis, leaf transectional symmetry and blade branching, as a context for these molecular genetic studies. Before turning to the specifics of these problem areas, I review the most fundamental features of plant morphological organization, especially in reference to the relationship of the cell and tissue levels of organization to that of the organism's morphology.

The Basic Organization of the Higher Plant Body

If we were to look for an example of the most fundamental model of higher plant morphology and organization, my choice would be the siphonous marine green alga *Bryopsis*. Despite its simplicity, *Bryopsis* embodies the fundamental fea-

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tures of higher plant organization. An individual “shoot system” exhibits a bipinnate organization, with a primary axis producing lateral branches, which in turn repeat this branching pattern (fig. 1A). Both primary and lateral branch axes have apical meristems at their extremities, where branches arise as lateral outgrowths in a typical acropetal succession (fig. 1B). A median optical section of the tip of an axis in *Bryopsis* illustrates what at first appears to be a fundamental difference from vascular land plants. Its plant body is an acellular tube whose multinucleate protoplast is not chambered into cellular subunits (fig. 1B). Hence, *Bryopsis* exhibits an uncoupling of morphogenesis from cell division. As a result, the initiation of lateral branches involves simply the outpouching of the delimiting cell wall (fig. 1B).

Since higher plants are obviously multicellular, how can *Bryopsis* be considered a model for them? Over the past 10 yr there have been several publications demonstrating the independence of patterns of cell division from form generation in vascular plants (Kaplan and Hagemann 1991, 1992; Cooke and Lu 1992; Kaplan 1992; Smith et al. 1996). As noted by Kaplan and Hagemann (1991), morphogenesis in vascular plants, like that in *Bryopsis*, also is a result of differential deformation of the outer common cell wall, and the division of cells internal to it follow this peripheral deformation. This concept is expressed in figure 2, in which different cellular configurations are drawn in the outline of the multinucleate, coenocytic *Bryopsis* (fig. 2A). In figure 2B, the pattern of cell lineages drawn is that of a typical uniaxial parenchymatic red alga, such as *Leveillia jungermannoides* (Kaplan and Hagemann 1991), whereas in figure 2C, the periclinal cell lineage pattern typical of vascular plants has been drawn in. This series of drawings underscores the independence of form generation from the cell partitioning patterns and, hence, the basis for the relative independence of the morphology of a plant from its anatomy. It is also the reason that these organisms exhibit convergences in morphology while preserving diagnostic histologies.

Bryopsis also is an effective model for vascular plants because its tubular branches can be considered the equivalents of higher plant organs (e.g., leaves). In this context, our concept of plant organs is that they are regional outgrowths of the plant body whose principal role is surface expansion and the positioning of the functional units (i.e., specific cell and tissue types) in a particular configuration in space for their effective roles. Using this most general and basic concept of plant organs, we do not distinguish between leaf and lateral branch because in the shoots of flowering plants, aside from their distinctive and diagnostic positional relationships, these two organs can converge with one another functionally (e.g., leaves or flattened branches [phyloclades] [Troll 1937–1943]).

This fundamental conception of higher plant organs harkens back to the views expressed by Hofmeister (1868). In his characteristically insightful way, Hofmeister designated all of the so-called organs of a plant shoot (lateral branches, leaves, and hairs) as outgrowths or *Sprossungen* (Hofmeister 1868). He used this more general term because he realized that the distinction between these different types of surface expansions was only a matter of degree. He understood that the differences in so-called plant organs reflected their status in the hierarchy

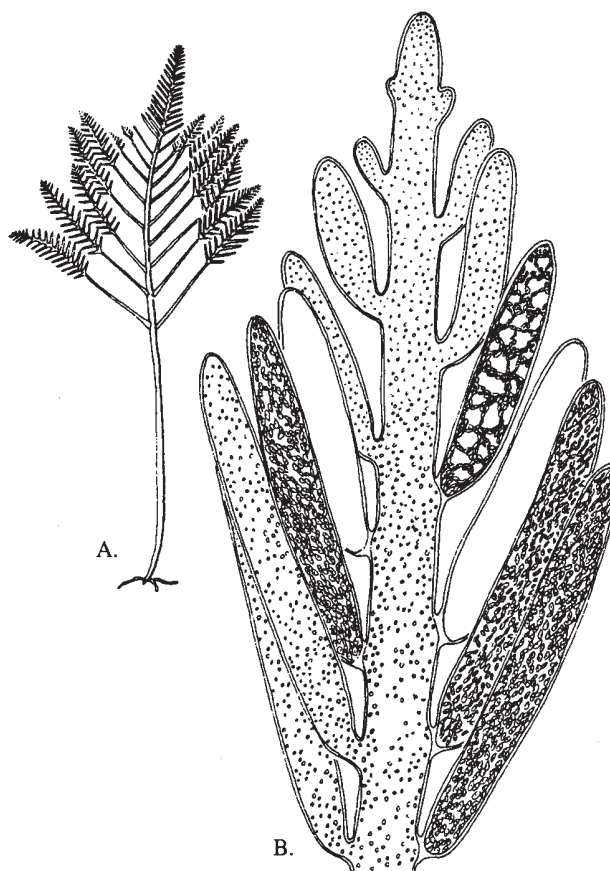


Fig. 1 Morphology of *Bryopsis corticulans*. A, Whole plant. B, Median optical section, showing the initiation of branches without concomitant cell division. Redrawn with permission from Smith (1955).

of the shoot as a whole, not marked qualitative differences in their structure and function, as is the case in animal organs.

In contrast with the more generalized lateral outgrowths of the plant body, which all can have the same general functions (photosynthesis, transport, transpiration, respiration, etc.) and cell and tissue types, animal organs are characterized by very specific functions (muscles, liver, heart, nervous system, etc.) and by an equally specific variety of cell and tissue types to carry out these multitudes of roles. Hence, it is natural for students of animal development to see linkages between organ development and cell differentiation in animals, whereas these functions are not linked in plants.

Again, with its uniformity of outgrowths, *Bryopsis* is a useful general model. As we will see, having such a basic model of shoot morphology allows the researcher to avoid a good deal of the unnecessary recent debate over organ homologies (Sattler 1974, 1988, 1990, 1992). As we will note at the end of this article, the *Bryopsis* model also provides a more realistic view of the relationship of genes to plant organ development. Nevertheless, while I emphasize the unifying general concept of *Sprossungen* for plant “organs,” it does not mean that I correspondingly accept the basic tenets of Sattler (1992) and

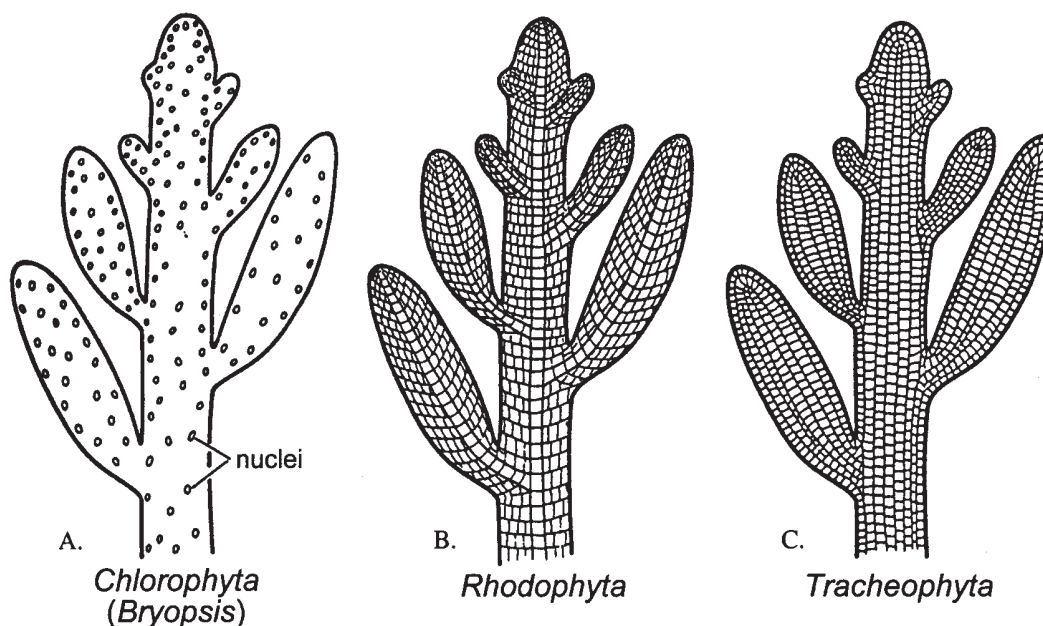


Fig. 2 Model of plant multicellularity in which all three plant types exhibit the overall shape of *Bryopsis* (A) but have their protoplasts secondarily chambered into cell lineage patterns of a uniaxial red alga (Rhodophyta) (B) and a vascular plant (Tracheophyta) (C). Reproduced with permission from Kaplan and Hagemann (1991).

Sattler and Rutishauser (1992). Unfortunately, the latter authors substitute these superficial similarities or convergences between “organ types” in plants for equivalences without realizing that the most fundamental properties distinguishing these *Sprossungen* from one another (leaf vs. shoot branch) depends on their respective position in the plant body as a whole, or what I refer to as the plant’s organization. It is these positional differences that account for the basic differences in transectional symmetry and for whether the particular structure is more or less determinate or indeterminate; it is these traits that are decisive in terms of the definition of these categories of outgrowth in plants, rather than their specific qualitative features.

Relationship of the Cell and Tissue Levels to the Morphological Level of Organization

Many of the recent articles on leaf development have implied, if not stated outright, that the cell and tissue levels of organization are the determinants of the morphology of the leaf and other plant organs. This is at least in part the result of the acceptance of animal organ concepts without the recognition of the fundamental differences between the features of plant and animal organization. It also is a reflection of the tacit acceptance of the conventional hierarchy between the cell and whole-plant levels of organization. According to that hierarchy, cells are aggregated into tissues, tissues are aggregated into organs, and organs are aggregated into organ systems, which form the whole plant (fig. 3, scheme I). Such a hierarchy reflects the long-standing assumption that the cell is the building block of the organism (Kaplan and Hagemann 1991). However, this viewpoint is contradicted by the evidence avail-

able for plants (Kaplan and Hagemann 1991; Cooke and Lu 1992).

From the perspective of the pathway of development in plants, the sequence is just the reverse of that inferred from the cell to organism hierarchy (cf. fig. 3, scheme II with scheme I). The primary event that occurs at the plant’s meristem is organ formation or morphogenesis. Histogenesis or tissue differentiation then follows morphogenesis (fig. 3, scheme II). Histogenesis is first manifested by the definition of the major tissue zones or so-called primary meristematic tissues (protoderm, ground meristem, and procambium) followed by the final phases of cell differentiation from the cell derivatives of these primary tissue zones (fig. 3, scheme II). According to this scheme, *Bryopsis* is simple because its development remains at the stage of morphogenesis at which organs are formed but with no subsequent histogenesis. For histogenesis to occur, the tubular protoplast of *Bryopsis* would have to be partitioned into cells (fig. 2B, 2C), which would then serve as the basis for the definition of tissue zones and cell types. Higher plants differ from *Bryopsis* only because they undergo these final stages of internal differentiation. Attempts to link genetics with plant development must bear these developmental priorities in mind if researchers are to effectively couple these facets of plant development.

One of the results of misunderstanding the developmental hierarchy in plants has been the tendency to confuse anatomical characters for morphological features in interpreting plant developmental mechanisms, especially in leaf mutants. The temptation to do this can be great because histological features can seem to provide sharper and more precisely defined boundaries than organ shapes. The problem is that relying on them can result in erroneous developmental interpretations; whereas

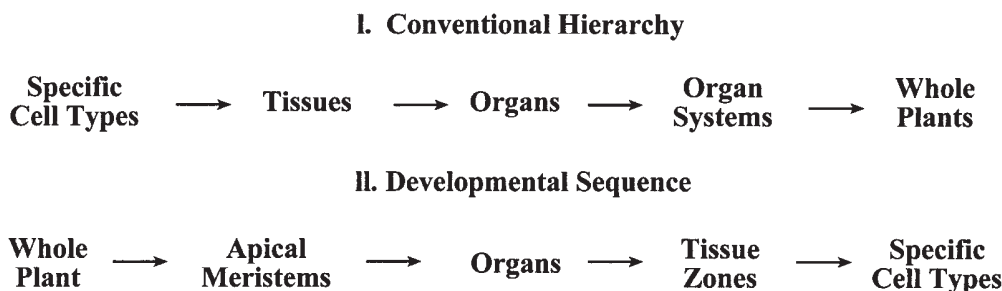


Fig. 3 Comparison of conventional hierarchy of the relationship of cell to organisms in plants (I) with the actual developmental sequence of the differentiation of these levels of organ zonation during plant development (II).

the anatomy may be determined by the morphology (Kaplan and Cooke 1997), the anatomy does not determine the morphology. For example, in developing leaves of *Isoetes andina*, the regional morphological differentiation of their leaf primordia into upper and lower leaf zones proceeds independent of their anatomical or internal differentiation (Kaplan, in press). Since this leaf's histology transcends these principal morphological regions, it is clear that the histological level of organization cannot serve as a marker for the basic morphological regions of this leaf.

A similar problem exists in Freeling's interpretation of the genetic control of leaf zonation in maize leaves (Freeling 1992). Traditionally, the leaves of corn and other grasses have been described as being differentiated into two major longitudinal zones: a distal blade and a proximal sheath (Troll 1937–1943). Traditionally, the ligule has been used to demarcate the boundary between these two zones (Troll 1937–1943). That the ligule is an anatomical and not a morphological marker is evident from the type of ligule polarity mutants that Freeling and his coworkers have described (Freeling 1992; Freeling et al. 1992). The seven ligule polarity mutants exhibit differing patterns of penetration of sheath tissue into the blade sector (see fig. 5 in Freeling 1992). In some (*Lg3-O* and *Lxm1-O*), sheath tissue projects into the median region of the blade, restricted either to the midvein or including the adjacent intercostal regions. In others (*Kn1-O*, *Lg4-O*, *Hsf1-O*, and *Rld1-O*), they are more lateral. In the mutant *Kn1-O*, sheath tissue jackets several lateral veins to either side of the midvein.

Freeling (1992, p. 52) characterizes these mutants as changing "the shape of the sheath blade boundary such that some sheath pushes into the blade," but examination of the mutant leaves confirms that it is their histogenesis rather than their morphogenesis that is affected. These mutations have effectively uncoupled the cell differentiation patterns from the morphological patterns. It is not that the sheath region has pushed into the blade region but rather that the distribution of sheath-type tissue has been altered as a result of the mutation process so that the blade regions are now mosaics of blade and sheath tissues. Morphologically, blade and sheath are the same as they are in the wild-type leaf.

These results demonstrate again the independence of the determination of the histological level from the morphological level of organization. These results merely reinforce the fact that the anatomical level of organization cannot be substituted for the morphological level in making judgments of develop-

mental mechanisms. The real morphological articulation in bifacial leaves of monocotyledons, such as *Zea*, is between the upper and lower leaf zones. Like many monocot leaves, the upper leaf zone in grass leaves is restricted to an inconspicuous precursor tip (*Vorläuferspitze*; Troll 1955), where the margins of the blade become confluent at the leaf apex (Kaplan 1973). Thus, both blade and sheath in such leaves are derived from the same basal zone of the leaf primordium and are, therefore, analogous and not homologous to the blades of dicotyledons. Given that these mutants represent simply tissue differentiation variants in the same developmental morphological zone of the leaf, it is not surprising that they do not display significant differences in leaf morphology.

These examples highlight the necessity of clearly distinguishing the levels of organization affected in mutant phenotypes before drawing conclusions on the developmental affects of specific mutations. I now turn to the application of these basic concepts with regard to the evaluation of two problem areas of leaf morphogenesis.

Leaf Transectional Symmetry

Principles and Problems

Of all the recently studied areas of leaf morphology and development, the one that has been at the greatest variance with information from plant morphology and the one that has produced the greatest terminological and conceptual confusion is leaf transectional symmetry. The article that stimulated interest in this area is the one that describes the *phantastica* gene in *Antirrhinum*, written by Waites and Hudson (1995). Unfortunately, this article also caused a number of interpretive problems as a result of the assumption and misapplication to a plant of terminology and concept systems derived from animal developmental biology.

In describing leaf transectional symmetry, Waites and Hudson (1995) used the term "dorsoventrality" to refer to this type of symmetry when they really mean dorsiventrality. Dorsiventrality is a category of transectional or rotational symmetry through which only a single symmetry plane can be drawn (Troll 1937–1943). It has also been termed "monosymmetric symmetry" (Endress 1999). The term "dorsoventral" has been used in animal developmental biology to refer to the occurrence of a developmental gradient between the dorsal and ventral sides of a structure. Thus, the term "dorsoventrality"

has developmental/mechanistic implications that are not implicit in the symmetry type. Because Waites and Hudson are referring to the transectional symmetry type, the proper term is “dorsiventral.” These authors also add confusion to their descriptions by mislabeling dorsal and ventral sides of primordial and mature leaves. In botanical nomenclature, the terms “dorsal” and “ventral” have been applied to leaves as if they were erect in posture. Hence, the term “dorsal” has been used to refer to the abaxial side or the side away from the axis because it reminded botanists of the back side. The term “ventral” has been used as a synonym for the adaxial side because it would correspond to the belly side of the leaf oriented toward the axis. Because the terms “dorsal” and “ventral” are only useful with reference to leaves that are predominantly erect in posture, the terms “adaxial” and “abaxial” have been preferred in designations of leaf morphology and orientation. Similarly, since the terms “upper” and “lower” are only meaningful for leaves that are horizontally oriented, adaxial and abaxial are also preferable to use to refer to the upper and lower surfaces of the leaf, respectively. Not only can the use of “dorsal” and “ventral” for the faces of a dorsiventral or bifacial leaf be confusing, but these descriptors become incomprehensible when used in the converse manner of Waites and Hudson (1995). It is more disturbing to see some of these errors being accepted uncritically by other workers and, hence, perpetuated (Martienssen and Dolan 1998; McConnell and Barton 1998; McHale and Marcotrigiano 1998; Timmermans et al. 1998; Tsukaya 1998).

Waites and Hudson and others imply that leaf primordia are radial or near radial at inception and secondarily become dorsiventral as a result of lateral growth (Waites and Hudson 1995; Martienssen and Dolan 1998; McConnell and Barton 1998; McHale and Marcotrigiano 1998; Timmermans et al. 1998; Tsukaya 1998). This premise is incorrect. Regardless of the ultimate transectional symmetry of a given leaf sector, all leaf primordia are dorsiventrally symmetrical from the time of their inception as lateral projections from the shoot apical meristem (Hagemann 1970; Kaplan 1975).

The fundamental dorsiventrality of leaf primordia can be seen especially clearly in the sections and scanning electron micrographs of primordia and young leaves in tobacco (*Nicotiana tabacum*) (Poethig and Sussex 1985). The adaxial side of the tobacco leaf primordium is flat, whereas its abaxial side, in continuity with the stem periphery, is rounded, accounting for the clear dorsiventrality of the primordia from the earliest stages of their protrusion. This dorsiventrality is expressed increasingly with leaf age, as the faster rate of trichome initiation on the abaxial side highlights the differential rate of maturation between these two sides of the tobacco leaf.

If a given leaf sector is going to become unifacial (i.e., appear virtually radial because it has only one face), it does so secondarily as a result of precocious adaxial meristematic activity, which serves to round out and, hence, eliminate the leaf's primordial adaxial surface to form what looks like a radial to near radial leaf sector. As emphasized by both Hagemann (1970) and Kaplan (1975), even though such a leaf sector may look radial in transection, it usually will show subtle signs of its basic dorsiventrality. Moreover, the transectional symmetry of given leaf zones will differ along the length of the leaf. For example, although many leaves may exhibit unifacial petioles,

their leaf bases and lamina regions are usually bifacial and, therefore, dorsiventral. The transectional symmetry is not indicative of the particular longitudinal leaf zone; a unifacial region may not be only a petiole homologue but in many monocotyledons can be a blade homologue as well (Kaplan 1973, 1975).

Aside from the aforementioned problems of the terminology of leaf transectional symmetry by Waites and Hudson (1995), it is difficult to sort out the developmental priorities and, hence, the sequences of processes in the development of leaf transectional symmetry and corresponding tissue development and distribution. The problem arises because the authors do not effectively separate morphogenesis from histogenesis. Since the descriptions of both Waites and Hudson (1995) and McConnell and Barton (1998) use the distribution of tissue types as the marker of the effect of their mutant genes, their articles seem to imply that it is the cell types that determine the leaf dorsiventrality. This may not have been intended, but it is a result of their presentation. Since we know that dorsiventrality is a property of the youngest leaf primordium, it is clear that the respective cell types cannot be determinants of the leaf's basic transectional shape. This principle becomes clear when we examine the range of variations in cell type distribution in dorsiventral (bifacial) leaves in the next section.

The Relationship between Leaf Transectional Shape and Photosynthetic Tissue Distribution

Figure 4 illustrates the principal variations of the relationship of leaf morphology to anatomy. In these diagrams, the leaf's morphology is illustrated by the transectional outline, whereas its anatomy is indicated by the distribution of its photosynthetic tissue (usually palisade tissue), illustrated by hatching within the blade's transectional outline. Thus, these figures

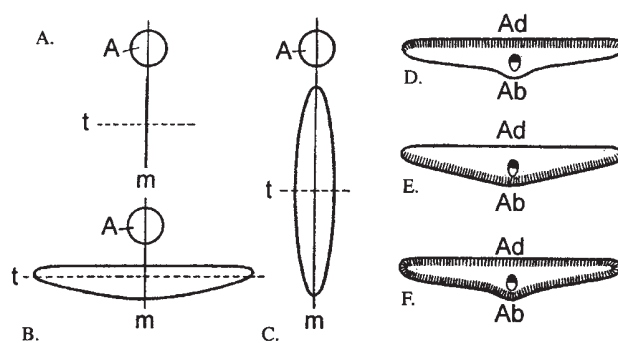


Fig. 4 Relationship of leaf anatomy to morphology. A, Principal planes of leaf flattening: A = axis, m = median plane, t = transverse plane. B, Leaf flattened in the transverse plane at right angles to the long axis of the shoot. C, Leaf flattened in the median plane of the shoot (i.e., parallel to the long axis of the shoot). D–F, Differences in photosynthetic tissue distribution in bifacial leaves. D, Normal bifacial leaf with assimilation tissue on the adaxial surface. E, Inversely bifacial leaf with assimilation tissue on the abaxial surface. F, Equifacial leaf with assimilation tissues on both adaxial and abaxial surfaces. Xylem tissue = black, phloem tissue = open, photosynthetic tissue = striped, Ab = abaxial side, Ad = adaxial side. Redrawn with permission from Troll (1937–1943).

indicate how blade histology may or may not be correlated with blade transectional shape or morphology.

As the plant's principal photosynthetic organ, most leaves are flattened organs that expose the maximum surface area for light interception and gas exchange. With reference to the shoot as a whole, leaves are flattened in one of two planes, the transverse plane (*t*, fig. 4A, 4B) or the median plane (*m*, fig. 4A, 4C). Most leaves have their blades flattened in the transverse plane, that is, at right angles to the long axis of the shoot (*t*, fig. 4B). By contrast, some species (e.g., *Iris*, *Acacia*, *Podocarpus*, and *Tmesipteris*) have their leaf blades flattened in the median plane (i.e., parallel to the long axis of the shoot; *m*, fig. 4C). Although median blade orientation can also come about through secondary torsion of a transversely flattened blade (conversion of the condition in fig. 4B into that in fig. 4C [e.g., in *Eucalyptus*]), the majority of species (*Iris*, *Acorus*, *Acacia*, and *Tmesipteris*) have vertically planated blades because their blades grow radially (i.e., along the median plane; fig. 4C). Such radial growth is the result of adaxial and intercalary meristems, which more typically are involved with rib and/or blade thickening (Kaplan 1970, 1973, 1975, 1980). Thus, they exhibit the transformation of volume growth into surface growth but surface growth at right angles to its usual (transverse) plane (fig. 4C).

The other illustrations in figure 4 are of transections through the blade regions with a typical bifacial transectional shape. Since both shoots and roots can also be dorsiventral (Troll 1937–1943), the term “bifacial” usually has been used to refer to dorsiventrally symmetrical leaves. Leaves are termed “bifacial” because in transection they have two different faces, each with a different shape, an adaxial face and an abaxial face (fig. 4D–4F). The vascular bundles in bifacial leaves have their xylem poles oriented toward the leaf's adaxial side and their phloem poles toward its abaxial side (fig. 4D–4F).

Although all of the leaf blade transections shown in figure 4D–4F are bifacial and have the same dorsiventral symmetry, they differ in the distribution of those mesophyll cells with the greatest chloroplast concentration (i.e., the palisade layers of the blade). For example, figure 4D is a transection of a normal bifacial blade with its photosynthetic palisade tissue located on its adaxial side. Figure 4E is a transection through an inversely bifacial blade with its palisade-like tissue on its abaxial side. The transection in figure 4F is cut through an equifacial leaf with its assimilation tissue located on both its adaxial and abaxial surfaces. Despite the similarity in the transectional outline of these bifacial leaf types, these differences in photosynthetic tissue distribution reflect the differences in orientation of each leaf type in space.

For example, leaves that are normally bifacial tend to be oriented horizontally so that their assimilatory tissue faces upward, toward the incident light. Inversely bifacial blades, however, are found in leaves that stand erect and lie appressed to the shoot axis so that only their abaxial surface is exposed to light. For example, certain succulent species in the Crassulaceae exhibit inversely bifacial tissue distribution. Typically, the stomata in such leaves are located on the leaf's adaxial surface. Here, they are covered by the overlapping leaves, reducing water loss in the arid environments in which this type of plant grows. Similarly, the leaf lobes of the aquatic fern *Azolla* are also inversely bifacial and lie appressed to the shoot axis with

only their abaxial photosynthetic tissue exposed to incident light (Kaplan and Groff 1995).

One of the most interesting examples of an inversely bifacial leaf is that found in the resupinate leaves of the genus *Alstroemeria*. Resupinate leaves are bifacial leaves that are twisted secondarily in their petiole regions. In the case of *Alstroemeria*, its assimilatory tissue differentiates on its abaxial rather than its adaxial surface (it is inversely bifacial as fig. 4E). However, each of its inversely bifacial leaves undergoes a torsion through 180 degrees so that its abaxial surface comes to face upward toward the incident light.

Finally, equifacial leaves are organs that grow erect so that incident light strikes the assimilatory tissue around the entire circumference of their blades. So-called window leaves in members of the ice plant family Aizoaceae are examples of equifacial leaves. Shoots of the South African stonecrop genus *Lithops* (Aizoaceae), for example, grow buried in the dry desert soil, with only the tips of their leaves exposed directly to light. The tips of the leaves of *Lithops* have a window region, devoid of chlorophyll, through which light passes, striking the chlorophyllous tissue on both adaxial and abaxial surfaces of the buried leaves (Krulik 1980). The equifacial distribution of assimilatory tissues provides an adequate photosynthetic surface while at the same time burying the stomata to reduce water loss.

From this survey of the relationship of leaf anatomy to morphology, it is clear that the two are separate and to some extent independent developmental features. Markedly different tissue distributions in leaves of identical transectional shape are only possible if the two facets of leaf development (morphogenesis and histogenesis) are independent of one another. These data alone put to rest any notion that cell types determine the morphogenesis of the leaf. They also emphasize that the broader aspects of whole plant morphology, including the spatial orientation of organs, must be taken into account in trying to understand how leaf form and function actually are controlled genetically and environmentally.

The Development and Morphology of Dissected Leaf Blades

A second area of leaf morphogenesis that has been a recent focus of plant molecular genetics concerns so-called compound leaves. Because dissected leaf blades offer the possibility of developing strikingly elaborated, branched blade surfaces in some of their mutants, this problem area has captured the interest of several research groups. Some of the mutants produced have raised legitimate questions with regard to the morphogenetic relationship of dissected to undissected blades and also the relationship of the former to whole shoots, which they can superficially resemble.

Relationship of Dissected to Undissected Blades

Leaves with elaborated blade surfaces traditionally have been called compound leaves (*folia composita* after Linneus). Doubtless the term originated because in such leaves, the segments (pinnae or leaflets) can be so individualized that each pinna resembles an individual simple leaf with its own stalk, in this instance called a petiolule (fig. 5D). Because of the

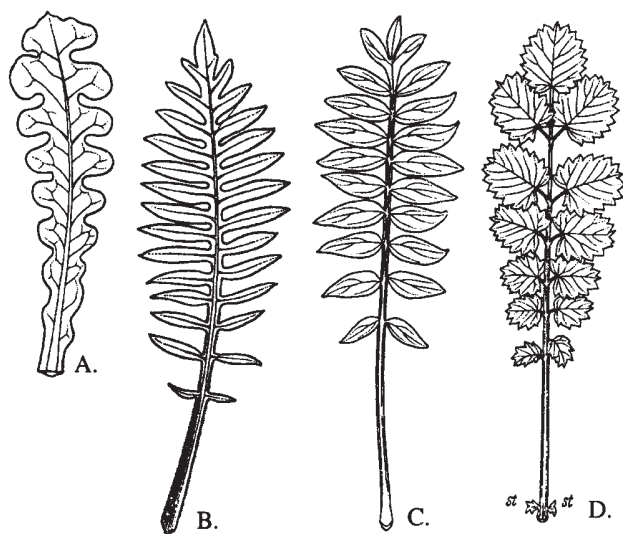


Fig. 5 Comparison of leaves with differing degrees of individualization of their segments. A, *Limonium sinuata*; B, *Serratula radiata*; C, *Polemonium coeruleum*; D, *Sanguisorba minor*; st = stipule. Redrawn with permission from Troll (1937-1943).

degree of resemblance of the subunits to whole simple leaves, such an elaborated blade could naturally be viewed as a compound structure, resulting from the union of many free, individual leaves. Of course, developmentally, the process is just the converse: the blade surface is subdivided into its subunits rather than being united from free parts.

And while the term “dissected blade” is a closer approximation to the developmental, morphological reality, it also implies developmental processes, which are not general for leaves with divided blades. For example, there are species whose leaf blades actually start out as unified, undivided structures but which become subdivided secondarily into segments, either by a process of localized tissue death (apoptosis) (e.g., *Monstera* [Kaplan 1984]) or by blade plication followed by tissue splitting (e.g., palm leaves) (Kaplan 1984). These plants are examples of true blade dissection.

The majority of leaves with elaborated blade surfaces initiate their subdivisions by what can be called a process of “blade branching.” Whether the segments become leaflets, marginal lobes, or marginal teeth in the mature leaf, they arise as free, lateral, lobelike protrusions from the embryonic margin of its leaf primordium (fig. 6C). Higher orders of blade branching occur when the pinnae themselves form segments or pinnules (fig. 6D). With such an iterative branching system, it then is possible to produce many orders of lamina branching, resulting in the highly elaborated mutant leaves produced in tomato (Hareven et al. 1996; Sinha 1997).

The justification for calling this process blade branching is that it occurs by the same processes as shoot branching, namely dichotomous branching or lateral branching, even though this type of branching occurs at a different level in the hierarchy of morphological organization (the leaf rather than the shoot level). The principal difference is that in shoots, branch origin is acrogenous (i.e., at the apical meristem), whereas in leaves,

the segments originate from the flanks of the dorsiventral primordium, the marginal meristem (fig. 6C). Dichotomously branched blades are found not only in juvenile leaves of ferns but also in select angiosperms as well (e.g., *Drosera binata* [Troll 1937-1943]). In the majority of divided blades, leaflet initiation occurs from the marginal meristem down the flanks of the young leaf and, hence, is clearly an example of lateral branching (fig. 6C, 6D).

Seeing leaf elaboration as the same process as occurs in the shoot is consistent with our *Bryopsis* model of shoot morphology, in which branching of the plant body as a whole is an iterative process of *Sprossungen* (Hofmeister 1868), initiated as lateral outgrowths (fig. 1B). The principal difference is that since the leaf primordium is dorsiventral, the branching process is confined to a single plane rather than along all radii. Thus, consistent with the *Bryopsis* model, the different degrees of elaboration of the outgrowths of the plant body depend on their symmetry properties in the hierarchy of the shoot and their corresponding degrees of determination, not on any unique properties in those respective positions.

Comparative leaf morphology in a great range of plant species has demonstrated the structural relationship between simple (unbranched) and branched blades (Troll 1937-1943). Not only can simple and dissected blades be found in heteroblastic series along the length of an individual shoot, but they can also be found between closely related species of the same genus. The theoretical diagrams in figure 7A-7C show that a palmately branched (fig. 7B) and a palmately lobed leaf (fig. 7C) have virtually identical primordia but differ in the distribution of growth in the regions of that common primordium. In a leaf with a palmately branched lamina, growth is predominantly in the free lobes and lesser in the central region (fig. 7A, 7B). By contrast, in the palmately lobed blade (fig. 7C), growth is favored in the central part of the primordium and

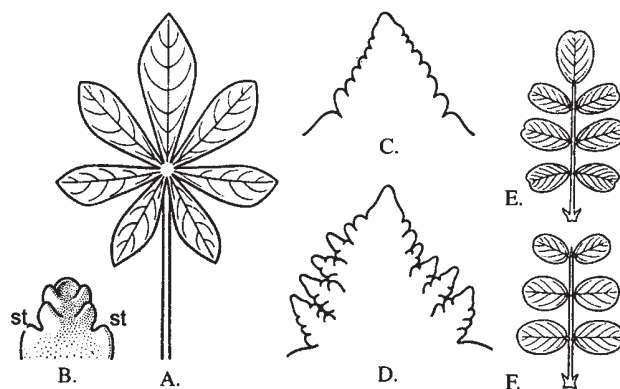


Fig. 6 The relationship of blade growth to different patterns of leaflet insertion on the leaf axis. A, Palmate or digitate blade in which all pinnae are inserted at a single point. B, Primordium of a leaf with a digitate blade, showing pinna inception without elongation of the leaf axis. C, D, Developmental stages of the twice pinnate leaf of *Carum carvi*, showing elongation of the leaf axis as the pinnae are initiated. D, Acropetal initiation of pinnules on each pinna. E, Imparipinnate leaf with a terminal pinna. F, Paripinnate leaf with rudimentary (unexpanded) terminal pinna. Redrawn with permission from Troll (1959).

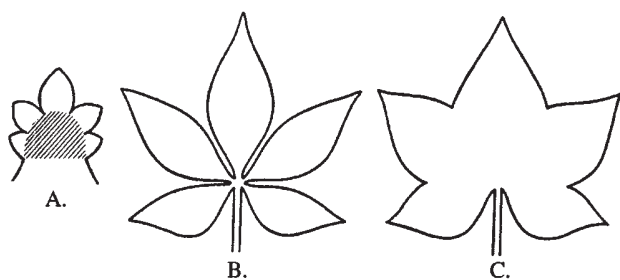


Fig. 7 Diagrammatic representation of the differences in growth distribution in leaf primordia of fully dissected and lobed leaves. *A*, Idealized leaf primordium with leaflet primordia in the form of marginal lobes. Central part of the primordium is striped. *B*, Fully dissected blade subdivided into distinct pinnae. *C*, Lobed leaf in which greater growth has occurred in the central part of the primordium than in the free lobes. Redrawn with permission from Troll (1937–1943).

is lesser in its marginal lobes (fig. 7A, 7C). Hagemann (1970) has shown that in different species of *Pelargonium*, the differences between pinnately lobed and pinnately dissected leaves are not just the result of the difference in positional distribution of growth but are also attributable to the relative timing of the difference in growth distribution. If the leaf is pinnately dissected, with fully individualized pinnae, then its free lobes are initiated earlier and show an earlier expansion, whereas if it is a pinnately lobed leaf, growth in the central region of the lamina occurs earlier, with the marginal lobes being initiated later and showing delayed expansion (Hagemann 1970).

Figure 5 shows a spectrum of leaf morphology proceeding from a pinnately lobed blade (fig. 5A) to one that is pinnatifid (i.e., pinnately cut) (fig. 5B) to one in which the leaflets are completely separate but sessile (no petiolule; fig. 5C) to one in which the pinnae have distinct petiolules, giving the impression that this is a compound leaf (fig. 5D). Here again, the clear morphological linkages between the members of this series result from a common developmental pathway. The morphological relationship between simple and dissected blades is summarized in figure 8. It is clear that both leaf types are equivalent morphologically, showing the same regional differentiation into leaf base, petiole, and blade (cf. fig. 8A, 8B). The main difference is that the blade region is divided into subunits, the pinnae in figure 8B, and unified in figure 8A. Nevertheless, total blade shape is the same in figure 8B as that seen in figure 8A, as indicated by the hatched line linking the apices of the pinnae in figure 8B.

Although the evidence of the structural relationship between leaves with branched and those with unbranched blades is clear and unequivocal, recent studies on the molecular-developmental aspects of these two leaf types have produced conclusions that have confused the issues. Again, the problems are largely the result of an inadequate context for the interpretation of their results. For example, Hareven et al. (1996) argued that since overexpression of *Knotted-1* gene (*Kn1*) in mutants of tomato produced increased ramification of its blade but did not produce dissected blades in simple blades of *Arabidopsis* or simple-leaved mutants of tomato or even corn (Sinha and

Hake 1994), leaves with simple and branched blades have different developmental programs.

This is not to say that there may not be differences in the development of these two leaf types at the molecular level but rather that information has no impact on the interpretation of morphological relationships between these two leaf types. Morphological questions can only be answered with morphological data. Furthermore, the lack of a comparable response to an exogenous application of a gene does not mean that the overall developmental relationships are different. It indicates, rather, that it is the morphological context that is regulating the expression of that gene and not the converse. In the case of maize leaves, as we have noted above, the so-called blade region is not a homologue of the blade region of dicotyledonous leaves (Kaplan 1973) and would thus be expected to respond differently. It would be more informative to study gene expression in a single plant with a heteroblastic change from simple to compound leaves, or vice versa, in order to determine the extent to which changes in leaf morphology are correlated with changes in gene expression, or what Hareven et al. (1996) have termed “developmental programs.”

Leaf or Shoot Homology for Pinnate Leaves?

Finally, we must consider a question that has been dealt with by plant morphology for its entire history: Are compound leaves homologous with leaves or shoots or both? Since molecular geneticists are producing mutants that can seem intermediate between simple and divided leaves, they are confront-

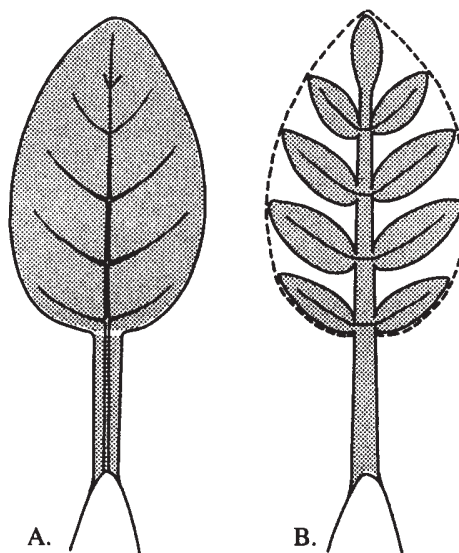


Fig. 8 The relationship between simple (unified) and dissected blades. *A*, Simple or unsegmented blade of a leaf differentiated into lamina, petiole, and leaf base. *B*, Leaf with a pinnately dissected blade in which the blade region is subdivided into subunits, the pinnae, or leaflets. Outline around the pinna apices indicates the equivalence of its divided blade to the undivided blade in *A*. Leaf has the same regional differentiation into lamina, petiole, and leaf base as leaf *A*. Upper leaf zone (lamina and petiole) shaded; lower leaf zone (leaf base) unshaded. Redrawn with permission from Kaplan (1975).

ing this question anew (Sinha 1997, 1999). Superficially, at maturity such leaves can resemble a dorsiventral shoot with a distichous phyllotaxis (figs. 5D, 6F), and this resemblance is increased by the delayed elongation or complete suppression of elongation of the rachis units converging with rosette shoots (fig. 6A) or shoots with elongated internodes (fig. 6E, 6F). Sattler and Rutishauser (1992) have repeatedly used examples like these to support their critiques of basic organ concepts. Unfortunately, Sattler and Rutishauser (1992) have focused their analyses on processes and disregarded the morphological organization, or context in which the processes are taking place. For example, they pay no attention to the suite of characteristics that define the leaf in the body of the plant as a whole (i.e., position in the shoot, basic dorsiventrality defining the locus of its meristem, the equivalence to simple leaves, etc.).

In her recent review of the molecular correlates of leaf development, Sinha (1999) similarly confuses processes for organization and, hence, gives credence to and accepts Sattler and Rutishauser's arguments. She cites the formation of adventitious apical meristems at the junction between the petiole and rachis axis in tomato mutants that fail to maintain the shoot apical meristem and axillary meristems as evidence that "the tomato compound leaf has some stem-like features and may be an intermediate structure between simple leaves and stems" (Sinha 1999, p. 439), while, at the same time, she cautions that this observation might not be generalizable.

Troll (1937–1943) has documented numerous examples of shoot initiation from leaf surfaces in ferns and angiosperms. Given that the pattern of such adventitious shoot production reflects the distribution of meristematic activity in the parent leaf, it is obvious that it is the leaf that controls the positions of bud production, not the transformation of a leaf into a shoot or the expression of an organ intermediate between leaf and shoot, as Sinha (1999) suggests. These types of interpretative problems reflect the need for an appropriate morphological context to effectively interpret the results from molecular genetics.

If we return now to our model plant *Bryopsis*, we can better

understand how these dilemmas in organ homology can arise. Looking at its overall morphology, *Bryopsis* exhibits a branched pinnate form reminiscent of a pinnate frond. In its most elemental state this pinnate shape comes about through the iterative production of outgrowths or *Sprossungen* (fig. 1A). These outgrowths are the most simplified equivalent of plant organs. Since each of these *Sprossungen* is determinate and has a limited existence because each will abscise, we could equate them with leaves, as Hofmeister (1868) did. Or, if we took a different perspective, we could see these lateral branch systems as the equivalent of pinnately divided, branched leaves (fig. 1A). No matter how morphologically differentiated the different orders of *Sprossungen* in higher plants may be, they reflect the same kind of ground plan we see in *Bryopsis*. However, in contrast with *Bryopsis*, successive levels of this organizational hierarchy in vascular plants (branches, leaves, and hairs) can clearly be distinguished from one another on both positional and qualitative criteria. For us, *Bryopsis* simply highlights that plant organography is of a different degree of differentiation than that observed in animals. In our search for genes controlling plant morphology, we may not find organ-specific genes, but rather we may find genes that modulate development in a general way. Certainly many of the plant genes characterized to date control histogenesis rather than morphogenesis. It is only when we come to grips with how plants are actually constructed and develop vis-à-vis animals that we will make progress in understanding their true developmental biology.

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