SHOOT AND COMPOUND LEAF COMPARISONS IN EUDICOTS: DYNAMIC MORPHOLOGY AS AN ALTERNATIVE APPROACH

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ABSTRACT

Recent developmental studies suggest that the compound leaf is a more or less incompletely developed shoot. Instead of considering leaves and shoots as non - homologous, this interpretation draws a continuum between leaves and shoots. This study considers the plant as a hierarchical series of units on which similar developmental processes are at work, and where each level (shoot, compound leaf, leaflet) is ‘repeated’ by the next higher level. Measurements related to the expression of developmental processes operating on leaves at the shoot level and on leaflets at the compound leaf level were used to determine if similar processes are at work at these different levels during early stages of organogenesis. Plants with compound leaves showing acropetal leaflet inception, representing a total 16 species from 10 eudicot families, were studied. Based on several types of quantitative analyses, there appears to be a continuum between so-called leaflets, compound leaves, and shoots in the species studied. This perspective, qualified as dynamic morphology, parallels the classical interpretation and is an alternative to it.

Keywords : compound leaf, continuum, leaf development, leaflet, shoot, process morphology, developmental genetics.

INTRODUCTION

Recent molecular and morphological evidence reveals striking developmental similarities between shoots and compound leaves as previously reported by Arber (1950) and others (Sattler & Rutishauser, 1992; Lacroix & Sattler, 1994; Lacroix, 1995; Hofer *et al.*, 1997; Poethig, 1997; Rutishauser & Sattler, 1997; Hofer & Noel Ellis, 1998; Veit, 1998; Sinha, 1999). One significant similarity is the initial orientation of the lateral elements perpendicular to the long axis of the structure on which they are inserterd. In this context, pinnately compound leaves resemble short distichous shoots with two, more or less opposite rows of leaves. Such resemblance occurs during early initiation of the leaflets of many species that develop leaflets from leaf base to tip (Rutishauser & Sattler, 1997).

Recent studies of leaf morphogenesis in the field of molecular biology support the idea of homologous developmental processes and origins between compound leaves and shoots (Hofer *et al*., 1997). Well characterized genes such as KN1 (KNOX family gene active in the shoot), UNI, and OSH1, affect the degree of indeterminacy (Smith & Hake, 1994) or complexity (extent of branching) of compound leaves in specific taxa (Sato *et al*., 1996; Hareven *et al*., 1996; Hofer *et al*., 1997; Fukuda, Yokoyama & Tsukaya, 2003). For example, transgenic tobacco plants over-expressing the *kn1* gene form shoots directly on the leaf surface (Sinha, Williams & Hake, 1993).

According to process morphology (Sattler, 1990; Jeune & Sattler, 1992; Sattler & Jeune, 1992; Sattler & Rutishauser, 1992), modalities of growth such as those referred to above (i.e. degree of determinacy and branching) and others as listed by Sattler (1990) are in operation at various levels of organization. As criteria, these modalities of growth can be as useful as those descriptors relating to symmetry and position in the context of classical morphology. It is important to note that all these criteria represent different yet complementary perspectives. In other words, continuum-based criteria are not meant to replace classical ones.

Looking at form as a combination of developmental processes appears to complement current molecular approaches to development. The KNOX family gene *kn1* example mentioned above suggests that two different structural plant categories or hierarchical levels (leaf and shoot) may share a common process in the elaboration of form (i.e. 'endogenous factors' common to both shoots and leaves). Sattler & Rutishauser (1997), in a recent review of the relevance of morphology and morphogenesis to botanical research, provide further examples from the field of molecular biology to support this. As well, they discuss the relevance of continuum and process morphology in the context of molecular biology by pointing out that defining the classical term 'leaf' from a molecular point of view may turn out to be as difficult as defining it morphologically. Recent studies or reviews related to the genetic basis of leaf development (Bharanthan & Sinha, 2001; Dengler & Tsukaya, 2001; Bharathan *et al*., 2002; Golz & Hudson, 2002) could easily be discussed in this context.

The growing body of evidence supporting the fact that so-called compound leaves and shoots may be difficult to delimit as mutually exclusive categories (Jeune & Sattler, 1992) does not in any way suggest that morphological categories, as defined by the classical criterion of relative position within the plant (Dengler & Tsukaya, 2001), are not useful or are obsolete. The question in many situations that leads to debate and differences in interpretation is what is the relevance of the leaf-shoot continuum? In this study, we propose to address this issue by examining a variety of taxa and quantifying specific growth parameters linked to developmental processes during early stages of morphogenesis.

Consequently, the aim of this study is to show that, based on selected measurable growth parameters, typical shoots, compound leaves, and leaflets during early stages of development support an interpretation based on process morphology (Sattler, 1990; Jeune & Sattler, 1992). The uniqueness of our approach lies in the comparison of these structures during their early stages of development. We therefore

propose an alternative to complement classical morphology.

MATERIALS AND METHODS

Taxa - - Specimens representing 16 species from 10 families were collected at the Fairchild Tropical Garden (FTG), Florida in March 1997. The selection of each taxon was based on the availability of vegetative material at the Garden at the time of collection and the ease and accuracy with which meristems could be dissected and measured. For the purpose of this study, each taxon is identified by a two or three letter code (Table 1). The FTG reference number for each plant is also listed in table 1. Voucher specimens of shoot tips have been preserved in fixative and stored in the laboratory of the first author.

In order to take comparable measurements from shoots, compound leaves, and leaflets, the species that were chosen are all characterized by typical leaves and leaflets with acropetal growth (type of growth also found in shoots) and by shoots, leaves and leaflets that produce individualized lateral elements.

Preparation of specimens - - Shoot tips from each species (Table 1) were fixed in a 1 :1 :9 solution of formalin-acetic acid-alcohol (F.A.A.) in the field, and later transferred and stored in 70% ethanol. At least five shoot tips from each species were dissected under a stereo microscope. Shoot apical meristems, compound leaf primordia, and leaflet primordia were dehydrated in a graded ethanol series and critical point dried using CO_2 as a transitional fluid in a model 28000 LADD critical point dryer. Specimens were mounted on stubs, grounded with silver paint, then coated with 300 Angstroms of gold-palladium using a Denton Vacuum Desk II sputter-coater. All samples were viewed with a Cambridge S604 scanning electron microscope (SEM) equipped with a digital imaging system (SEMICAPS®).

Measurements - -The following measurements were made on three representative samples of leaflets, compound leaves, and shoots for *Polyscias fruticosa* 'Plumata' (Pf), *Polyscias obtusa* (Po), and *Oroxylon indicum* (Oi), and on compound leaves and shoots only for all other species used in this study (Table 1). Measurements were not obtained for leaflets on most of the species because they do not produce lateral elements (i.e. lobes). The parameters used are identified by Roman numerals throughout.

I - angle of divergence (sensu lato): The angle between the mid-points of successive lateral elements (leaf or leaflet primordia) in relation to the center of the apical meristem (e.g. angle between leaflets 1 and 1' on Figure 1; angle between leaves 1 and 2 on Figure 3). From a dynamic morphological point of view, this angle has the same significance for shoots, leaves, and leaflets. It corresponds to the angle formed between the center of the apex and the intersection of the median line through the apical dome at the level of initiation of lateral elements, each representing the end point of a parastichy.

II - angle of insertion: The measurement of the angle representing the width of a primordium in relation to the center of the apical meristem (e.g. angle □ on Figures 1 and 3).

III – radial plastochron ratio (sensu lato): The ratio of the distance between a lateral element and the growing tip for two consecutive leaves or adjoining leaflet primordia. Example: Ratio of radial distance of leaf 1 to shoot tip / distance of leaf 2 to shoot tip (Fig. 3) or ratio of distance of leaflet 1 to leaf tip / distance of leaflet 2 to leaf tip (Fig. 1).

IV – dorsiventrality (departure from circularity): The ratio of the smallest radius to the

largest radius (ratio of A to B on figures 1 and 3. As shown in figure 1, the base of the entire leaf primordium was used to calculate the long and short radii whereas the first 4 or 5 leaf primordia (or the outline of their bases once removed) were used to outline the diameter of the shoot from which the short and long radii were measured. We are aware of the risks involved in chosing a ratio of two problematical variables that are normally or nearly normally distributed. However, since these variables cannot be independent, the ratio cannot be a Cauchy variable.

V and VI - branching dynamics: Parameters of the linear regression [$Ln(Y)=a+bX$] between the logarithm of the longitudinal distance from the apex to a lateral element (Y), and the rank of that element (X). This relationship is based on the formation of elements at a regular rhythm during an exponential phase of growth or lengthening.

The ratio Y2/Y1 (corresponding to X1=1 and X2=2) is similar in proportion to the plastochron ratio defined above.

V - intercept (a): height of the apex. This parameter is therefore related to the logarithm of the rate of growth in length of the organ varying linearly with time.

VI - slope (b): density of lateral elements. This parameter is related to the rhythm of formation of lateral elements as a function of time.

Examples of the measurement of the distances from the apex to lateral elements are highlighted by broken dotted lines on figures 2 and 4. Parameters V and VI are considered an important measure of the dynamics of growth. For example, if the rhythm of the formation of lateral elements increases (i.e. increase in VI), the cells of the apex will consequently be used up more quickly (i.e. V will decrease) and vice

versa.

To confirm the appropriateness of using indirectly measured parameters V and VI, we conducted simulations where measured values of variances for the parameters in question were replaced with higher or lower estimates. This did not change the pattern of distribution of points obtained using PCA.

VII – height of the free apical dome: Distance from the apical meristem to the top of the youngest lateral element (leaf or leaflet primordia). Example: Y on figures 2 and 4.

To demonstrate the existence of a continuum, measurable variables that, a priori, could be discriminant were chosen. For example, shoots are typically radial in symmetry in comparison to leaves and leaflets; we would therefore expect our parameter IV, a measure of dorsiventrality or departure from circularity to reflect this distinction between shoots and leaves. Similarly, the rhythm of formation of lateral elements (parameter VI) or the height of the free apical dome (parameter VII) can also be considered as different for shoots, leaves, and leaflets.

Unavoidable errors of measurement crop up when two dimensional photos representing three dimensional structures are used. However, it can easily be shown that, in our case, the corresponding increase in residual variance remains negligible in relation to the variance associated with organs or species for each of the 7 parameters that were used. To demonstrate this, the total variance was broken down into its 'component parts' by using a Two-way ANOVA (Table 3).

Analysis - - Two types of complementary statistical analyses were used: (1)

cluster analyses (aggregative clustering and K-means clustering; used to verify the stability of the results), and (2) principal components (PCA) and discriminant analyses (DA) (Lebart, Morineau & Fénélon, 1979, Lebart, Morineau & Piron, 1995; Saporta, 1990).

These analyses are methods that are used to group individuals and the groups they form based on their respective distances. From a geometrical perspective, the distances between individuals (shoots, leaves, and leaflets), in the space within which they are represented, highlight degrees of similarity (affinities). We chose a Euclidian distance measure (PCA will be associated to such a measure) and adopted either the Ward method (based on the techniques of analysis of variance) or unweighted pair group average (UPGMA) for grouping individuals. These methods were chosen because they provide results that conform best to the typological approach.

Principal components analysis is best suited for quantitative data sets (individuals x characters). Discriminant analyses and cluster analyses are used to assess the extent of a continuum between shoots, compound leaves, and leaflets. In fact, if the three categories of organs (shoots, leaves, leaflets) are discontinuous, these types of analyses should show distinct groupings representing the three types of organs.

These two types of analyses are therefore well suited to examine the morphological values of shoots, leaves, and leaflets. If in fact we are dealing with objectively distinct entities as suggested by their specific designations and as confirmed by classical typological morphology, measurements representing shoots, compound leaves and leaflets should appear as three separate clouds of data points on all graphs

particularly in the case of the discriminant analysis and hierarchical trees. If this is not the case, and the three clouds of data points are confluent, a morphological continuum between the three entities known as shoots, compound leaves, and leaflets is more likely the case. This notion of continuum does not imply that we would be unable to distinguish between shoots, compound leaves, and leaflets, but that these apparently distinct terms may instead correspond to different levels of morphological differentiation based on dynamic variables, i.e. variables related to development (Jeune & Sattler, 1992; Sattler & Jeune, 1992).

To complement our general analyses, specific Discriminant Analyses were performed. Shoots, compound leaves, and leaflets were compared two at a time to determine which measured parameters would be the most pertinent in distinguishing between these structures. This analysis was done on the three species for which measurements were available for shoots, compound leaves, and leaflets (Pf, Po, Oi). For all other species, we compared shoots and compound leaves only (see above section on measurements).

All analyses were completed with Statistica v.5.5 and 6.0 (Statsoft Inc., Tulsa) and Statitcf v.4.0 (ITCF Boigneville) software.

RESULTS

Cluster analyses

K-means clustering: We were not able to identify three homogeneous (pure) groups corresponding to the typological approach. Instead, we were dealing with at least 4 groups. If we start with the assumption that we are in fact dealing with 4 groups, K-means clustering will generate the 4 most homogeneous groups by successive iterations based on 4 randomly chosen mobile centroids. Once this operation is performed, we find:

a) leaves (Ab, Ai, Cht, Fu, Gr, Oi, Po, Ti)

b) shoots (Ab, Ai, Cet, Cht, Eu, Fu, Gr, Ha, Kp, Mh, Mk, Oi, Ti)

c) leaves (Ai, Cet, Eu, Fu, Gr, Ha, Kp, Mh, Mk, Mt, Pf, Po)

and leaflets (Oi, Pf, Po)

d) shoots (Mt, Pf, Po)

When we choose a grouping in three classes, we obtain a, b+c, and d.

Hierarchical tree : Using the Ward method we found exactly the same grouping, either with four groups (a, b, c, d) or three (a, b+c, d). This is not surprising because the Ward method rests on minimising the variance like K-means clustering. A different, potentially more appropriate, method or aggregation (linkage rule) known as (unweighted pair-group average : UPGMA) was also used. To clarify the presentation of our results and to reduce the variability associated with individuals, we replaced the 3 measurements that were taken per variable for each species by their average value (Fig. 8).

The result is similar to the previous one, even though there is only one point per organ per species on figure 8:

a) leaves (Ab, Ai, Cht, Gr, Oi et Ti),

b) shoots (Ab, Ai, Cet, Cht, Eu, Fu, Gr, Ha, Kp, Mh, Mk, Oi et Ti)

and leaves (Cet, Eu, Fu, Ha, Kp, Mh, Mk, Mt, Pf et Po),

c) leaflets (Oi, Po et Pf),

d) shoots (Mt, Pf et Po).

It is interesting to note that these results are remarkably stable, consistent and support the idea of a continuum. The only observable difference, depending on the algorithm used, is in the position of the leaflets which are either associated with leaves or shoots.

When the linkage distances decrease, we observe a progressive breaking up of groupings of shoots, leaves, and leaflets (at a distance of approximately 60 units) for groups b and c. At this distance, there are three typologically distinct groups (Fig. 8):

1) sub-group of b (shoots),

2) sub-group of b (leaves),

3) group c (leaflets),

At higher values of linkage distances (90 units), it is impossible to separate the three types of organs completely and we have organs that are closer to other types of organs than their own type. These results support the idea of a continuum.

Principal Component (PCA) and Discriminant (DA) Analyses on entire data - - Based on the assumptions inherent in these types of analyses, the distribution of points

representing individual shoots, compound leaves, and leaflets on Figures 9 and 10 shows a morphological continuum between these three structures. The first three axes of the PCA account for 81% of the total variance (50% + 19% + 12%). Only the first two axes have a variance superior to that of the initial variables (14%). Since the DA is an analysis performed on the centroids of the three groups (shoots, leaves, and leaflets), only two axes are available. The pattern of a continuum obtained from the analyses does not imply that shoots, compound leaves and leaflets are indistinguishable but shows that there are similarities between them at these different levels of morphological differentiation. The bundle of vectors corresponding to correlations between the principal axes and the initial variables are represented on the graphs of our PCA and DA analyses. Since certain of these vectors are difficult to distinguish visually, tables with the numerical values have also been included (Tables 4 and 5).

Results from the Discriminant Analysis where the measure of the distances between individual points within each group (shoots, compound leaves, leaflets) are minimized, while the distances between groups are maximized show a similar if not more apparent pattern of continuity between these groups (Fig. 9) than the Principal Component Analysis (Fig. 10A,B). Nonetheless, in both cases, the projections show confluence between groups.

In the Discriminant Analysis (Fig. 9) and the first two axes of the principal component analysis (Fig. 10A), general trends are observed as far as they relate to the values of the measured parameters. These are indicated on the figures as converging lines denoted by Roman numerals. In the Discriminant Analysis, the values of parameters I (angle of divergence), II (angle of insertion), III (plastochron ratio), and VI

(representation of the density or packing of lateral elements) increase from the bottom to the top of the graph (Fig. 9). On the other hand, parameters IV (dorsiventrality), V (representation of the height of the apex), and VII (height of the free apical dome) show a decrease from the top to the bottom of the same graph (Fig. 9; Table 4). In the principal component analysis, the parameters form similar groupings in the plane of the first two axes (Fig. 10A; Table 5). Parameters I (angle of divergence), II (angle of insertion), III (plastochron ratio), and VI (representation of the density or packing of lateral elements) show an increase from the right to the left of the graph, parameters V (representation of the height of the apex) and VII (height of the free apical dome) from the left to the right, and parameter IV (dorsiventrality) shows a decrease from the top to the bottom of the graph (Fig. 10A). The representation of the PCA data in the plane of axes 1 and 3 (Fig. 10B) shows that the confluence or continuity between the three groups of points as observed in the plane of the first two axes is not an artefact of projection. The planes of axes 1 and 2 of the DA and PCA therefore allow for an accurate interpretation of the relationships between shoots, leaves, and leaflets. Leaves differ from shoots based on the relatively strong values of variables V (representation of the height of the apex) and VII (height of the free apical dome) and weak values of variables I (angle of divergence), II (angle of insertion), III (plastochron ratio), and VI (representation of the density or packing of lateral elements); the opposite situation is true of shoots (Fig. 10A). Leaflets, on the other hand, assume average values for all these variables. However, leaves and shoots have relatively higher values for variable IV (dorsiventrality); this distinguishes them from leaflets for which values associated with this variable are somewhat weak (as for variable V [representation of the height of the apex]).

Specific Discriminant Analyses - - Shoots, compound leaves, and leaflets for three species (Oi, Pf, Po) were compared in a pairwise fashion to determine which parameters best distinguished between these basic morphological categories.

A comparison between shoots and compound leaves (Fig. 11A) reveals that parameter I (angle of divergence) and parameters V and VI describing branching dynamics (i.e. relationship between the apex and the density or packing of lateral elements) best distinguished between compound leaf and shoot. This trend was also observed for the comparison between shoots and leaflets (Fig. 11C).

A comparison between leaves and leaflets (Fig. 11B) reveals that dorsiventrality (parameter IV) as well as branching dynamics (parameters V and VI - see above) were the most useful to distinguish between these types of structures.

Summary Data - - Figure 12 is a summary of the data in the form of a Discriminant Analysis for compound leaves and shoots within a species. Points representing the three leaves and three shoots that were measured were linked for each species represented on the graph. Assuming that a theoretical value of –1 corresponds to a shoot and a value of +1 corresponds to a leaf, it is easy to verify if the specific elements or organs that were measured have a value corresponding to that of their group. Results distinguish three types of taxa. In the first group (Po, Kp, Ti, Gr, Ab, Oi, Fu), shoots and leaves are distinctly separate. In the second group (Mt, Pf, Mk, Cht, Ai), one of the elements (leaf or shoot) has intermediate characteristics. In the third group (Cet, Hs, Eu, Mk), both elements have intermediate characteristics.

The three plants for which the leaflets were measured (Po, Pf, Oi) belong to the groups of plants where the distinction between shoots, leaves, and leaflets is clear.

This is shown graphically in figure 10A where the three clusters of black symbols representing shoots, leaves, and leaflets are well separated.

Although shoots and leaves were visually recognisable in all our examples (cf. Figs. 5-7), differences between these structures, based on the comprehensive number of parameters that were measured, show that this perception is not as clear during the initial stage of initiation of lateral elements. This means that differences between classical morphological categories are not as clearly delineated from a developmental point of view.

DISCUSSION

Based on the developmental parameters that were measured at early stages of development, there appears to be a continuum between leaflets, compound leaves and shoots; the differences between these morphological categories are not mutually exclusive. In other words, it is obvious that leaflets, leaves, and shoots have different characteristics but there appears to be no qualitative differences between these categories (Figs. 9,10,12). The fact that we are dealing with a continuum does not mean that categories are non-existent from the perspective of classical morphology. It's simply another point of view.

When average values for each parameter by organ type are consulted (Table 2), the following trends can be observed. Leaflets, leaves, and shoots can be distinguished by their angle of divergence sensu lato (parameter I). Leaves and leaflets on the other hand are closely related as far as angle of insertion sensu lato (parameter II), plastochron ratio (parameter III), and density or packing of lateral elements (parameter VI). Average values for leaves and shoots are similar based on dorsiventrality (parameter IV). Leaves stand out as far as the distance from the growing point to the top of the youngest element is concerned (parameter VII). However, the variance is such (25 times greater than for leaflets and leaves) that the average value itself is not reliable as an estimate of that parameter. It is important to note that these average values alone do not represent the full potential of each parameter as a discriminant variable. Only the more complete analyses that are presented in the form of discriminant factorial and principal components analyses take the variability of the averages and measurements into account.

In fact, our discriminant analyses (Fig. 11) confirm that parameters relating to branching dynamics (parameters V and VI), represented by the linear regression between the logarithm of the distance from the apex of a structure to a lateral element and the rank of that element, are important distinguishing factors for those three species (Table 1; Pf, Po, Oi) where shoots, compound leaves, and leaflets were compared. Measurements relating to dorsiventrality (parameter IV) and angle of divergence sensu lato (parameter I) also fall in that category. The fact that there are observable differences between morphological categories is in itself not novel or unique. Additionally, structures represented in figures 1to 7 are not different or unique to other systems that have been reported in the literature. This is intentional on our part because we wanted to analyse elements belonging to three morphological levels that were as 'typical' as possible and from which we could measure a specific number of growth parameters during early stages of development. Even though leaflets, leaves, and shoots have different characteristics, their distinction as mutually exclusive categories from a qualitative point of view is not possible unless classical positional information is used (Fig. 12). Our continuum-based approach therefore forces or exposes the limitations of the classical approach. However, it is not meant to invalidate the use of classical categories.

Results from this quantitative analysis are supported by earlier studies based on morphological observations of compound leaves and shoot systems at early stages of development (Sattler & Rutishauser, 1992; Lacroix & Sattler, 1994; Lacroix, 1995; Rutishauser & Sattler, 1997). This quantitative analysis is also supported by recent studies in molecular biology showing that similar genetic processes are operating at

these different morphological levels (Hofer *et al*., 1997; Poethig, 1997; Hofer & Noel Ellis, 1998; Veit, 1998; Sinha, 1999; Fukuda *et al*., 2003)

The parameters that were used in this study (and several others) together specify form or, as Sattler (1990) states, “form is process”. The approach or perspective of looking at form as a combination of developmental processes common to all developing structures makes the idea of a continuum between shoots, compound leaves and leaflets more plausible and is independent of the way these elements are classified for practical reasons. Our results show that the boundaries between classically defined categories of organs are not clearly delimited (Fig. 12). Even homeosis (the assumption by one part of an organism of the features of another part), a phenomenon that has received a lot of attention from a developmental and molecular genetic point of view (e.g. Sattler, 1988; Smith & Hake, 1994; Sattler & Rutishauser, 1997; Kramer, Dorit, & Irish, 1998; Sinha, 1999), appears to be more compatible with the idea of a continuum.

Our observations and those of others who suggest that there is a continuum between compound leaves and shoots are in agreement with some of the most recent studies in molecular biology and genetics as outlined in the introduction. From this perspective, a plant is not viewed as a juxtaposition of typologically different elements but as a nesting of partially similar units; leaflets are small leaves forming the compound leaf, the compound leaf itself represents a small shoot system, and shoots are small plants that together form the whole plant.

This way of looking at plant morphology is closely related to computer simulations of plant construction (Prusinkiewicz & Hanan, 1989) based on the use of recurrent ‘rules’ and also the theory of fractal geometry (or self-similar nesting

'emboîtement autosimilaire') of Mandelbrot (1982). At a recent conference, Mandelbrot (2000) stated that fractals can be viewed as forms where the detail reproduces the part and the part reproduces the whole « les fractales sont des formes telles que, indépendamment des sens que l'on donne aux mots, le détail reproduit la partie et la partie reproduit le tout ». He illustrates his point further with the use of a botanical example by explaining that branches of a tree are themselves little trees «les branches de l'arbre [sont] elles-mêmes de petits arbres complets » (Mandelbrot, 2000). This interpretation is similar to Goethe's (Arber 1946) who, during the early days of plant morphology, stated very generally that compound leaves are similar in form to shoots: «In a sequence of several leaves, the midrib is carried progressively further into the lamina; the fan-like simple leaf becomes torn and divided; and the end is a highly complex leaf, vying with a branch». Our study is based more precisely on the ideas Arber, whose contributions and their relevance to modern plant morphology were highlighted at a symposium at the International Botanical Congress in Saint-Louis in 1999. The proceedings of this symposium were published in a recent issue of the Annals of Botany. Our study supports the view that "compound leaves can be seen as intermediate between simple leaves and whole shoots" (Sattler & Rutishauser, 1997), or that "morphological variation in structures within an individual plant can be interpreted as reiteration of design" (Hofer, Gourlay & Noel Ellis, 2001), or that a "compound leaf can repeat the developmental pathway of the whole shoot, at least to some degree" (Rutishauser & Isler, 2001), and finally that "the part can be fully understood only in the context of the whole" (Kirchoff, 2001). All these ideas are attributed to and were developed by Arber in The Natural Philosophy of Plant Form (1950) where she "attempted to describe all structures as processes" and paid attention to "repetitive

branching", "differential growth" and "parallelism" (Classen-Bockhoff, 2001).

It would be erroneous to assume that we want to substitute the classical viewpoint with our idea of dynamic morphology. We prefer not to enter the debate over choosing one model over another (Fisher, 2002; Timonin 2002) but instead view dynamic morphology as a perspective complementing the traditional one. Using the best applicable model under specific circumstances seems to us to be more appropriate and in this context it is encouraging to see some type of conceptual convergence between our model and other disciplines (mathematics, computer science, and molecular genetics) as they apply to plants.

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Table 1. Species examined in this study.

Family	Code	Taxon	FTG reference
Araliaceae	Pf	**Polyscias fruticosa* Harms. 'Plumata'	64456
	Po	**Polyscias obtusa* Harms	X1641
Bignoniaceae	Kp	*Kigelia pinnata* DC.	P2192B
	Mh	*Markhamia hildebrandtii* Sprague	93144A
	Oi	**Oroxylon indicum* Vent.	N/A
Fabaceae	Mt	*Millettia thonningii* Baker	6183C
	Ti	*Tamarindus indica* L.	95753A
Meliaceae	Ai	*Azadirachta indica* A. Juss.	70405A
	Cet	*Cedrela toona* Rottler	X1210A
	Cht	*Chukrasia tabularis* A. Juss.	77436A
Myrtaceae	Eu	*Eugenia uniflora* L.	N/A
Oleaceae	Fu	*Fraxinus uhdei* Lingelsh.	1219A
Oxalidaceae	Ab	*Averrhoa bilimbi* L.	63130B
Proteaceae	Gr	*Grevillea robusta* A. Cunn.	93336A
Rutaceae	Mk	*Murraya koenigii* Spreng.	77708A
Sapindaceae	Ha	*Harpulia* cf. *arborea* Radlk.	X1213A

* species for which measurements for shoots, compound leaves, and leaflets could be obtained (see materials and methods under subheading 'measurements' for explanation)

N/A – not available

Table 2. Average values (and variances) for measured parameters.

	parameters						
	I	II	III	IV	V	VI	VII
Leaflets	83.79	58.49	1.40	0.53	2.92	0.18	19.6
	(258.10)	(482.29)	(0.009)	(0.027)	(0.389)	(0.006)	(228.91)
Leaves	106.63	54.17	1.27	0.78	5.08	0.13	138.91
	(616.33)	(200.04)	(0.016)	(0.031)	(0.343)	(0.002)	(4773.59)
Shoots	154.43	137.47	1.66	0.79	4.34	0.47	51.19
	(411.87)	(4379.1)	(0.300)	(0.034)	(0.170)	(0.058)	(391.654)

Table 3. Two-way ANOVA of three species for which measurements of all variables for shoots, leaves, and leaflets are available. The probability is the risk of falsely rejecting the null hypothesis H_0.

	Probability (species)	Probability (organ)	Probability (species • organ)
Parameter I	0.65%	0.00%	0.00%
Parameter II	0.00%	0.00%	0.00%
Parameter III	4.71%	0.00%	0.91%
Parameter IV	1.09%	0.01%	73.02%
Parameter V	0.07%	0.00%	0.74%
Parameter VI	0.31%	0.00%	15.92%
Parameter VII	0.09%	0.00%	0.37%

Table 4. Correlations between discriminant axes and variables (see also Fig. 9)

	Axis 1		Axis 2	
	Correlation	Cos^2	correlation	Cos^2
I	0.8816	*0.7772*	0.4724	*0.2232*
II	0.7283	*0.5304*	0.6855	*0.4700*
III	0.6063	*0.3676*	0.7955	*0.6328*
IV	0.8795	*0.7735*	-0.4756	*0.2262*
V	0.3874	*0.1501*	-0.9218	*0.8497*
VI	0.6915	*0.4781*	0.7227	*0.5223*
VII	-0.0925	*0.0086*	-0.9957	*0.9915*

Table 5. Correlations between principal axes and variables (see also Fig. 10)

	Axis 1		Axis 2		Axis 3	
	Correlation	*Cos²*	correlation	*Cos²*	correlation	*Cos²*
I	0.7121	*0.5070*	-0.2524	*0.0637*	-0.0804	*0.0065*
II	0.8505	*0.7234*	-0.2854	*0.0814*	0.0031	*0.0000*
III	07144	*0.5104*	-0.1651	*0.0273*	-0.3749	*0.1405*
IV	0.0501	*0.0025*	-0.5410	*0.2927*	0.8119	*0.6592*
V	-0.5387	*.02902*	-0.7291	*0.5316*	-0.2660	*0.0708*
VI	09101	*0.8284*	-0.2147	*0.0461*	-0.0755	*0.0057*
VII	-0.7204	*0.5190*	-0.5138	*0.2640*	-0.2876	*0.0827*

FIGURE LEGENDS

Figures 1-7. Scanning electron microscope (SEM) photographs of representative specimens of shoots, compound leaves, and leaflets. Figures 1-4. SEM of leaf and shoot specimens of *Azadirachta indica* (Ai) showing how measurements were taken. Fig. 1. Top view of young compound leaf. Scale bar = 100μm. Fig. 2. Side view of young compound leaf. Scale bar = 100μm. Fig. 3. Top view of shoot tip. Scale bar = 167μm. Fig. 4. Side view of shoot tip. Scale bar = 100μm. Figures 5-7. SEM of shoot, compound leaf, and leaflet of *Polyscias obtusa* (Po). Fig. 5. Shoot apex (arrow). Scale bar = 500μm. Fig. 6. Compound leaf with newly initiated lateral elements (bulges). Note presence of leaf sheath (arrow). Scale bar = 125μm. Fig. 7. Leaflet primordium also with lateral elements (bulges). Scale bar = 125μm. Symbols : A, distance from center to shortest side ; B, distance from center to longest side ; Y, height of free apical dome ; □, angle of insertion of a lateral element ; ascending sequence of Arabic numerals corresponds to the sequence of initiation of lateral elements from youngest to oldest.

Figure 8. Tree clustering (Euclidian distances, Linkage rule: UPGMA method) showing 4 distinct groups at a distance of 90 units. Box a, leaves; b, shoots and leaves; c, leaflets; d, shoots. Last letters of codes refer to leaf (L or Lf), shoot (S), or leaflet (Lt); preceeding two or three letters refer to species code (see Table 1).

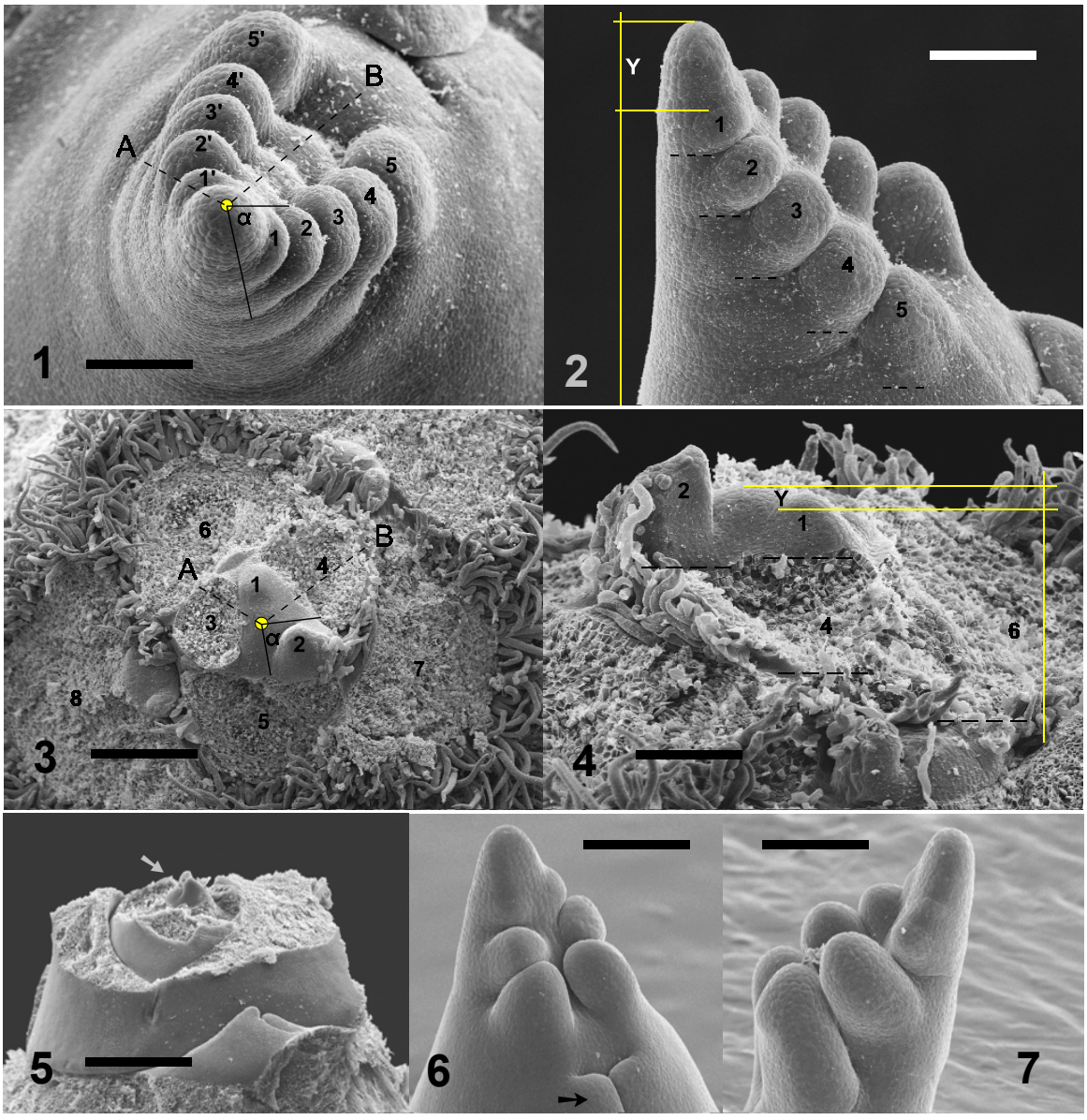
Figure 9. Discriminant Analysis (DA). Individual shoots are represented by circles, compound leaves by squares, and leaflets by triangles. The solid symbols represent the three species for which shoot, compound leaf, and leaflet measurements are available (see Table 1) whereas open symbols represent species for which shoot and

compound leaf measurements only are available. General trends as far as they relate to the values of the measured parameters are shown as diverging lines denoted by Roman numerals I-VII. Each segment indicates the direction of increasing values for each variable.

Figure 10. Principal Components Analysis (PCA). A. Representation of the data in the plane of axes 1 and 2. B. Representation of the data in the plane of axes 1 and 3. Individual shoots are represented by circles, compound leaves by squares, and leaflets by triangles. The solid symbols represent the three species for which shoot, compound leaf, and leaflet measurements are available (see Table 1) whereas open symbols represent species for which shoot and compound leaf measurements only are available. General trends as far as they relate to the values of the measured parameters are shown as diverging lines denoted by Roman numerals I-VII. Each segment indicates the direction of increasing values for each variable. The first two (or three) letters of each code refer to species (see Table 1) while the letters that follow represent shoots (s), leaves (l), and leaflets (lt). The number at the end of each code represents one of three samples for different organ types for each species.

Figure 11. Specific Discriminant Analysis. Data for the three species (Oi, Pf, Po) for which measurements are available for shoots, compound leaves, and leaflets are used in this analysis. The species for which leaflet measurements are not possible are excluded from this analysis. A. Comparison between shoots and compound leaves. B. Comparison between compound leaves and leaflets. C. Comparison between leaflets and shoots. In each case, the three most discriminant variables are listed beside each graph.

Figure 12. Discriminant analysis representing the three leaves and shoots measured for each species. Each species is therefore represented by six data points linked by one line. The dotted line represented by the value –1 is the ordinate of the centroid of the shoots and +1 represents that of the leaves. Three groups of species are highlighted in this figure: group A (Po, Kp, Ti, Gr, Ab, Oi, Fu), where shoots and leaves are distinctly separate; group B (Mt, Pf, Mh, Cht, Ai), where one of the elements (leaf or shoot) has intermediate characteristics; group C (Cet, Hs, Eu, Mk), where both elements have intermediate characteristics.


1
5'
4'
3'
2'
1'
A
B
α
1
2
3
4
5
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Y
1
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4
5
3
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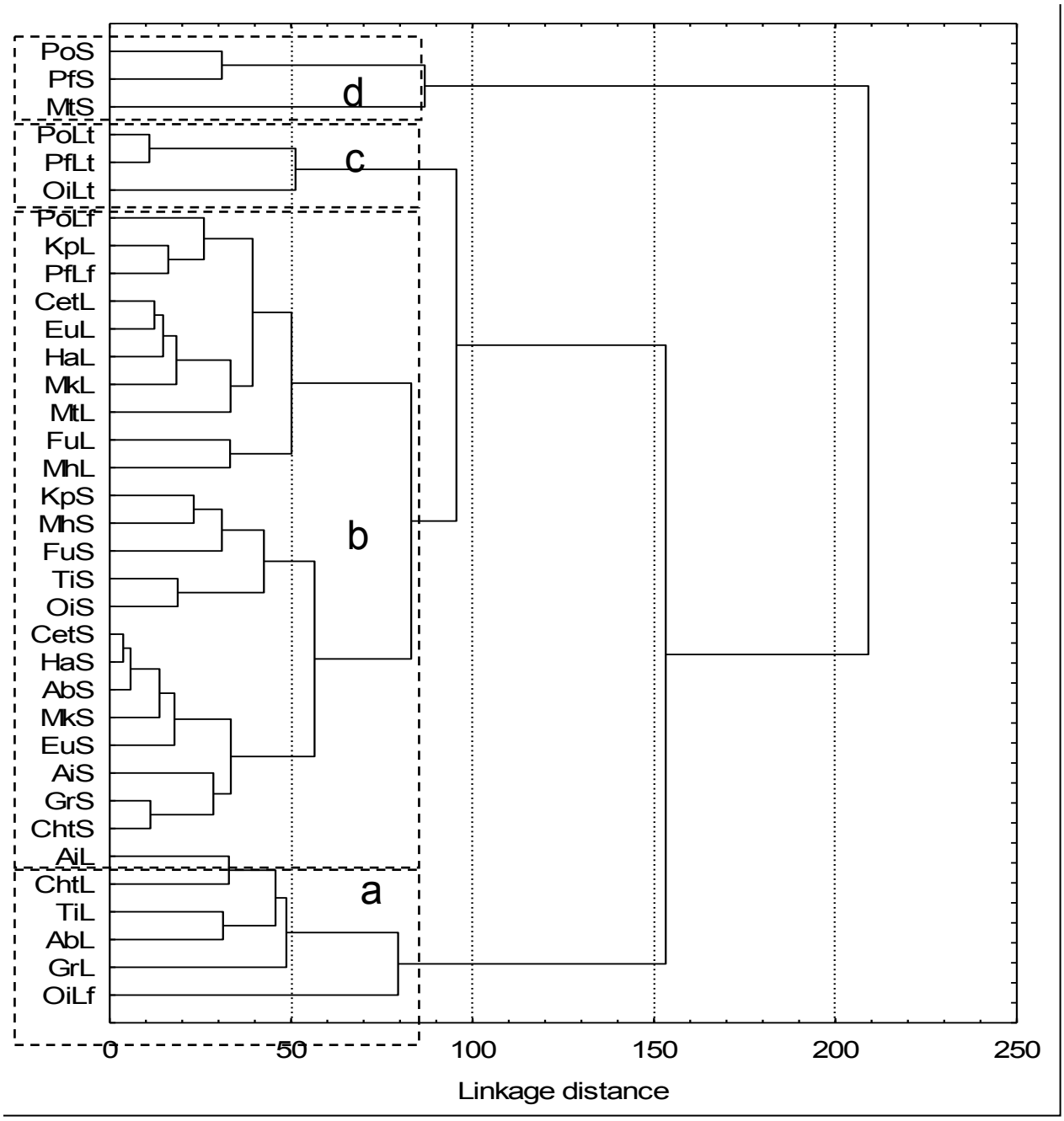
7


PoS
PfS
MtS
d
PoLt
PfLt
OiLt
c
PoLf
KpL
PfLf
CetL
EuL
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MkL
MtL
FuL
MhL
KpS
MhS
FuS
TiS
OiS
b
CetS
HaS
AbS
MkS
EuS
AiS
GrS
ChtS
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a
0
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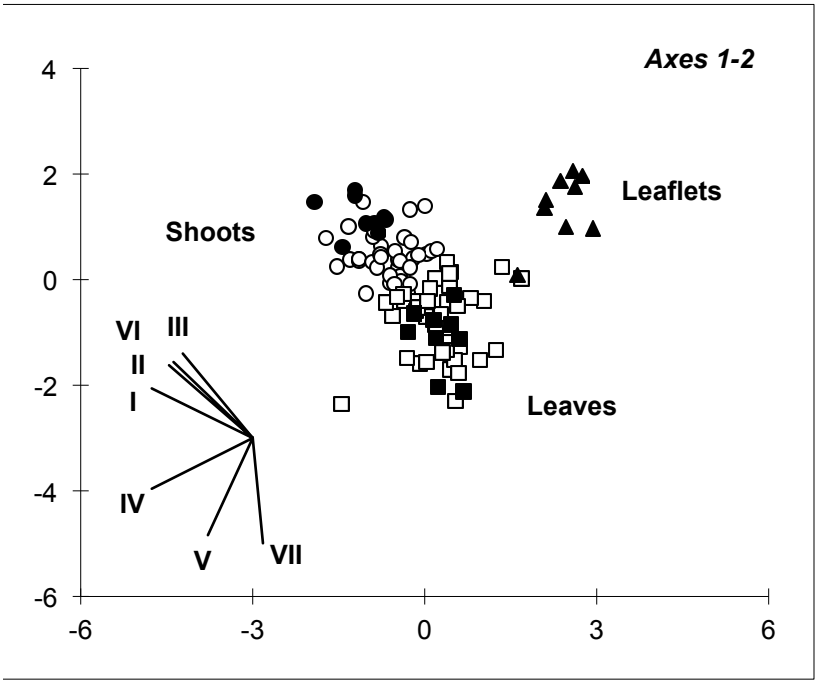
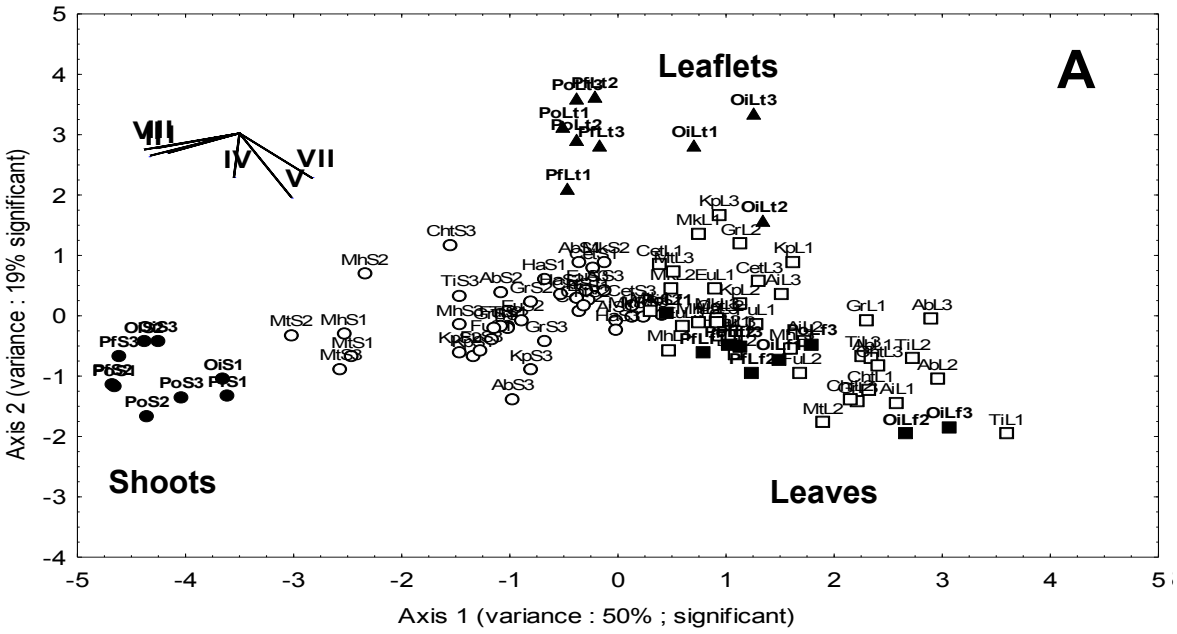
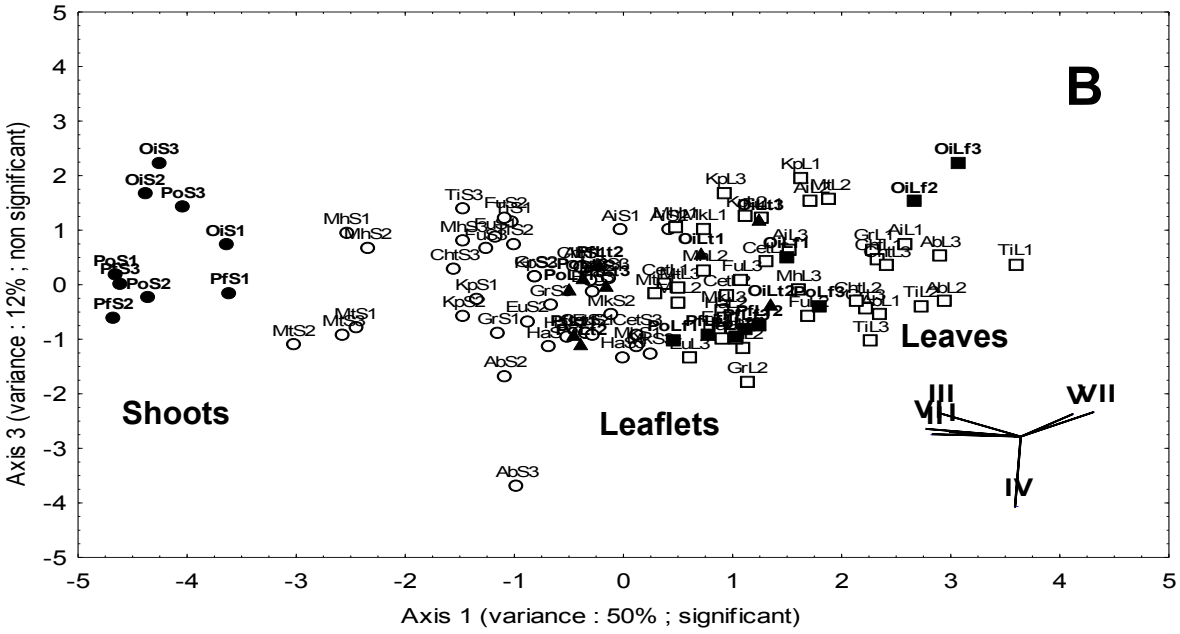
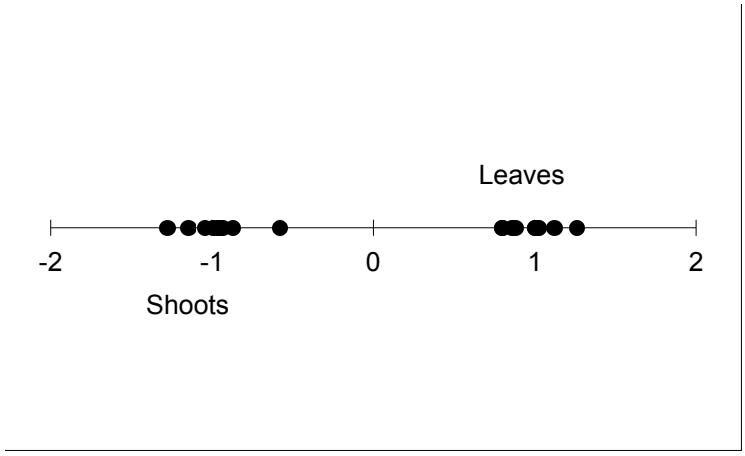
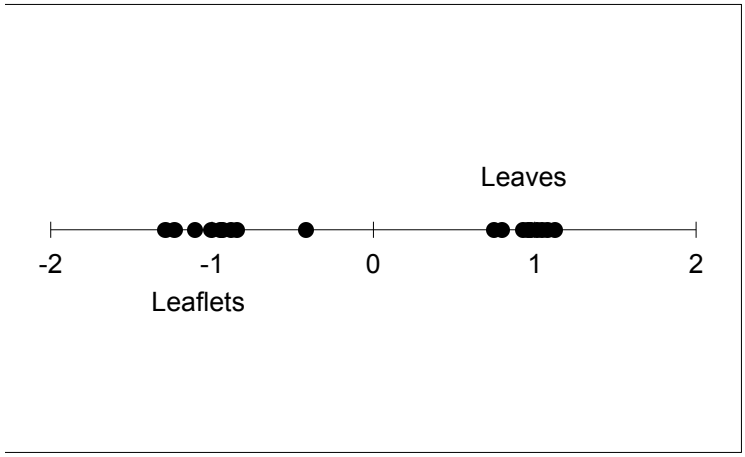
Linkage distance

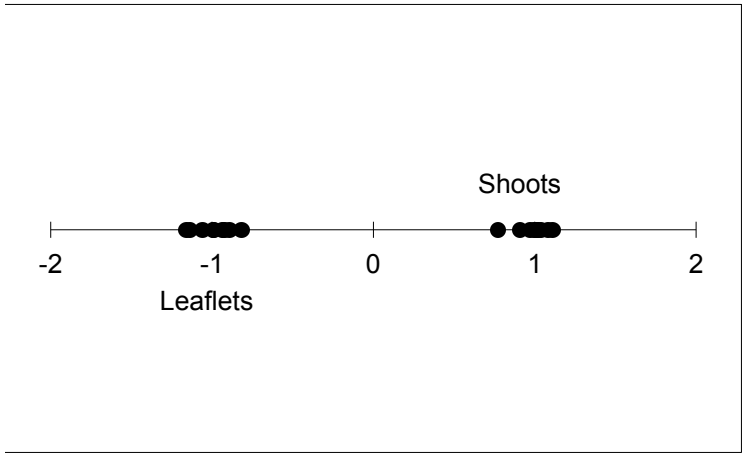

Axes 1-2
4
2
0
-2
-4
-6
-6
-3
0
3
6
Leaflets
Shoots
Leaves
VI
III
II
I
IV
V
VII

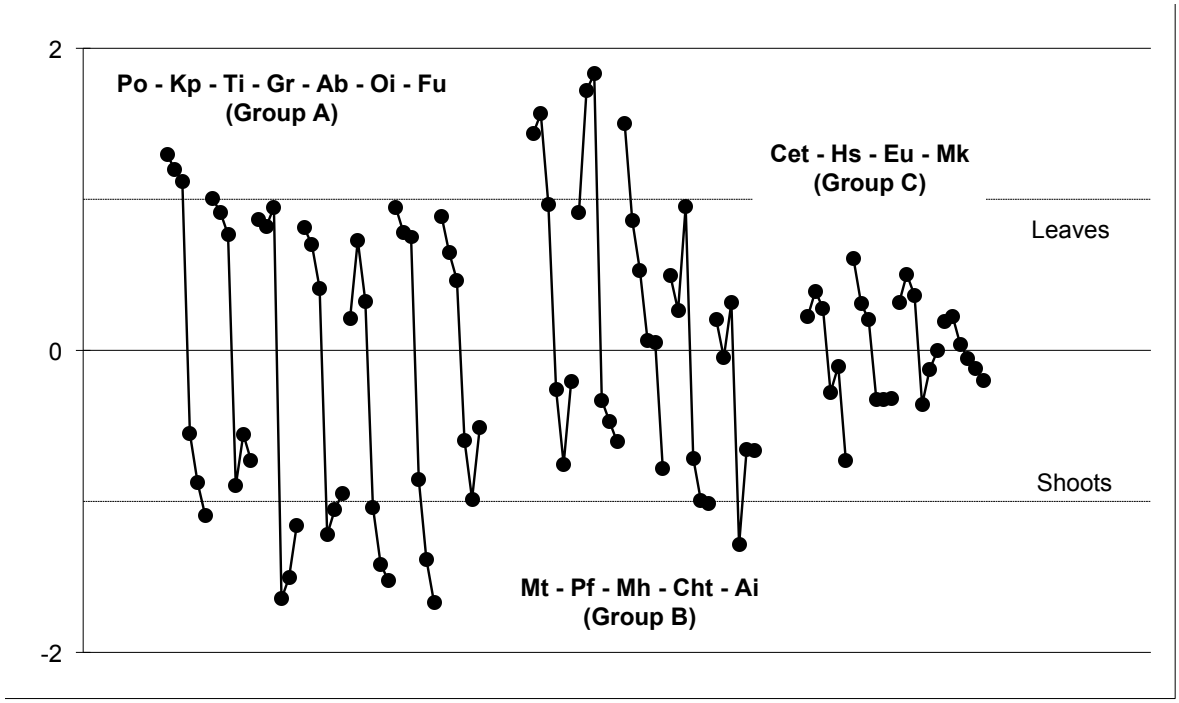

A
Leaflets
Shoots
Leaves
Axis 2 (variance : 19% significant)
Axis 1 (variance : 50% ; significant)
VIII
IV
V
VII
OiLt3
OiLt1
PfLt1
OiLt2
OiS1
PoS3
PfS1
PoS2
MhS2
MtS2
MhS1
MtS1
AbS3
OiLf2
OiLf3
TiL1


B
Shoots
Leaflets
Leaves
Axis 3 (variance : 12% ; non significant)
Axis 1 (variance : 50% ; significant)
OiS3
OiS2
PoS3
OiS1
PoS1
PfS3
PoS2
PfS2
PfS1
MhS1
MhS2
MtS2
MtS1
AbS2
AbS3
OiLf3
OiLf2
TiL1
VIII
III
VII
IV


Leaves
-2
-1
0
1
2
Shoots


Leaves
-2
-1
0
1
2
Leaflets


Shoots
-2
-1
0
1
2
Leaflets


2
0
-2
Po - Kp - Ti - Gr - Ab - Oi - Fu
(Group A)
Cet - Hs - Eu - Mk
(Group C)
Leaves
Shoots
Mt - Pf - Mh - Cht - Ai
(Group B)