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Homoeosis and shoot construction in *Azara microphylla* Hook. (Flacourtiaceae)

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SUMMARY

Each node of the dorsiventral shoot of *Azara microphylla* appears to have two leaves, a large and a small one, inserted on the same side of the stem. These have previously been interpreted as a leaf and a leaf-like stipule. There is a small, partly glandular, stipule-like structure at the outer edge of each pair of 'leaves', and one between the two 'leaves'. Developmental and anatomical studies suggest that there has been homoeotic replacement of the upper stipule by a 'leaf'. One of the small stipule-like structures, that at the outer edge of the larger 'leaf', appears to represent a normal, if reduced, stipule. The other two appear to be additional stipular structures related to the presence of homoeotic 'leaf'. Both the 'leaves' receive traces direct from the stem vasculature in a leaf-like manner. There are some structural anomalies which appear to have no functional significance and which may have arisen as side-effects of the homoeotic process.

Key-words: Azara microphylla, development, homoeosis, leaf, stipule.

INTRODUCTION

The unusual external morphology of the shoot of *Azara microphylla*, and other members of the genus, has occasionally attracted attention. On casual inspection, the shoot appears to have two entire leaves, a large one and a small one, attached at each node. According to Warburg (1894) at each node there is normally a leaf, a leaf-like stipule, and a small stipule. Dormer (1944) interpreted the shoot as having leaves in distichous phyllotaxis, each leaf having a single leaf-like stipule towards the upper face of the dorsiventral shoot. There seem to have been no anatomical or developmental studies.

In the family as a whole, most of the genera have simple leaves inserted singly, commonly with the normal complement of two stipules. The stipules are often small and caducous, or sometimes replaced by glands (Warburg 1894; Hutchinson 1967). The norm of shoot construction in the family is therefore quite ordinary, and *Azara* represents a dramatic departure from the norm. The structure and development of the shoot in *A. microphylla* has now been studied in greater detail and the results are presented here.

MATERIALS AND METHODS

Shoots of *A. microphylla* were collected in May 1990 from the Manchester University botanical gardens, and fixed in FAA fixative (Johansen 1940). At this stage shoots were actively extending and initiating leaves. Material for sectioning was dehydrated by standard methods and embedded in L. R. White resin; serial sections were cut at 3 µm using glass knives, and stained with toluidine blue. Developmental morphology was studied using epi-illumination light microscopy (Sattler 1968; Posluszny *et al.* 1980; Charlton *et al.* 1989). Whole shoots were stained in 0·5% acid fuchsin in 90% ethanol, or in 0·5% fast green in 80% ethanol, differentiated and dissected in 95% ethanol and stored in 100% ethanol. Material stained with fast green was further stained with acid fuchsin before photography.

OBSERVATIONS

In order to describe concisely an unusual morphological situation, it is helpful to encode the description within some generally understood frame of reference. In this case, as an *ad hoc* starting point, we can take the three morphological categories which have been noted in Flacourtiaceae with more conventional shoots (i.e. 'leaf', 'stipule' and 'gland') and use them, in quotation marks, as identifying labels for the structures in *Azara* which, superficially at least, fall into these categories. The most immediate result of this *ad hoc* approach is that the leaf and leaf-like stipule of earlier authors become the large and small 'leaves'.

Organography

The evergreen shoots of *A. microphylla* grow horizontally or obliquely, and present a dorsiventral appearance, with four ranks of shortly petiolate entire, sparsely toothed, dorsiventral 'leaves', with the large 'leaves' forming the two lower ranks (Fig. 1a and b). On close examination, each node (i.e. point of attachment of a 'leaf' pair) reveals more structures than earlier descriptions would suggest (Fig. 1c and d). There are three small structures attached at the lateral edges of the 'leaf' base, one at the outer edge of each petiole, and one between the two. These structures have a glandular tip, a basal region bearing plain trichomes and sometimes distinct lateral glands (Fig. 1e). Apart from the presence of lateral glands they are radial in symmetry (Fig. 1b and e). On our *ad hoc* basis they are identified as 'stipules'. There is a single bud inserted above the adjacent edges of the two petioles of a pair. There are a number of 'glands' attached to the adaxial faces of the petioles (Fig. 1c and d), and the few teeth of the 'leaf' margin are also glandular (Fig. 1f). There are numerous trichomes on the stem and petiole, fewer on the lamina, although the 'leaf' tip has a tuft of trichomes (Fig. 1g). The surface of the stem and the 'leaf' base region tends to be smeared with resinous material from the 'glands'.

The 'leaves' and 'stipules' eventually absciss individually leaving separate scars; the 'stipules' absciss well before their associated 'leaves'. There are no distinctive cataphylls in terminal or lateral buds, but the first 'leaves' of lateral buds are usually considerably reduced in size.

The vascular system

The internode has a vascular cylinder rather than separate bundles (Figs 1d, 2a and c). At each node the two leaves and the associated bud each receive a single vascular trace (Fig. 2a and b). There is a leaf gap above the point of departure of each 'leaf' trace, but the gap above the trace to the small 'leaf' extends only a short distance vertically (Fig. 2b). The bud trace is attached to the edge of the 'leaf' gap of the larger 'leaf', on the side nearest the small 'leaf'. It actually differentiates later than the leaf traces (Fig. 2c and d). The 'stipules' and 'glands' are not vascularized.

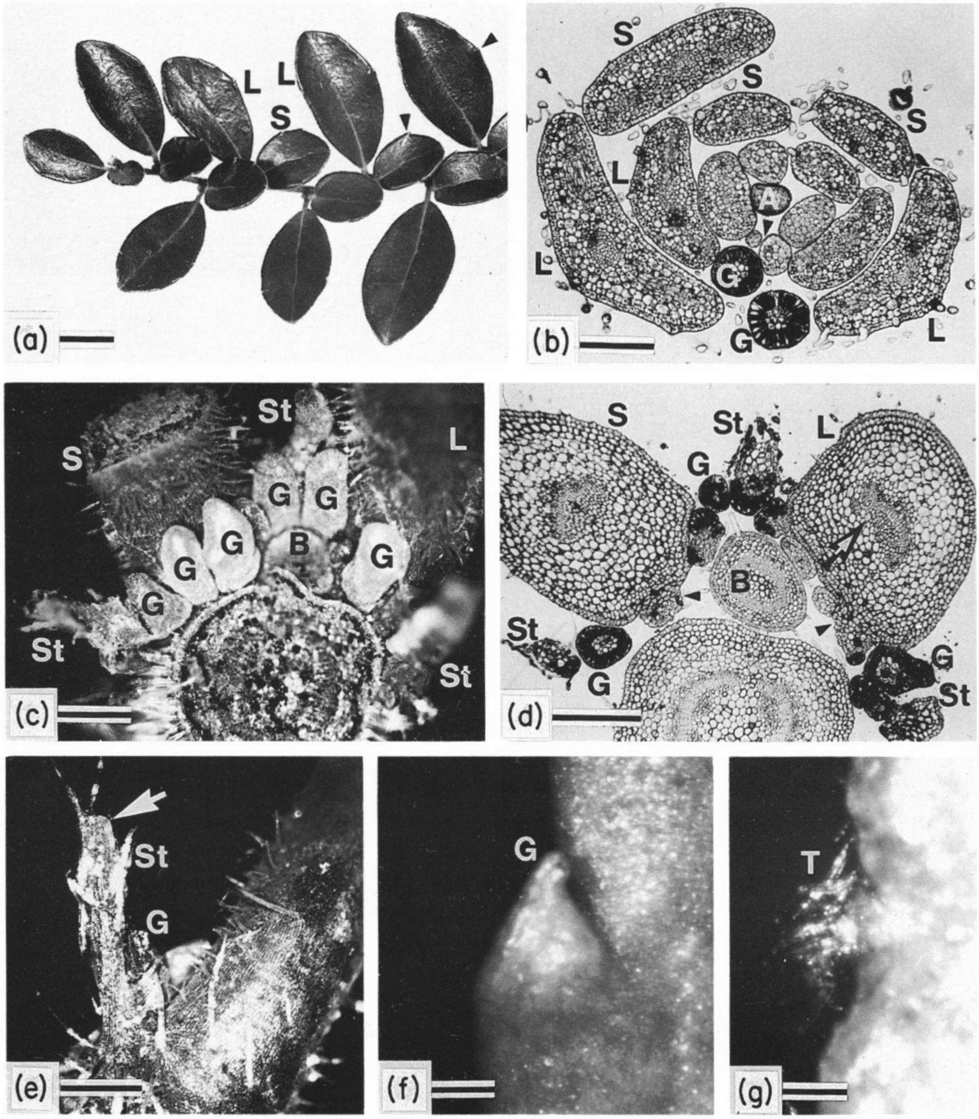

Fig. 1. (a) Shoot of *A. microphylla* showing large (L) and small (S) 'leaves'. Two leaf teeth are indicated by small arrowheads. Bar = 0·5 cm. (b) Transverse section of bud showing shoot apex (A), two ranks of large 'leaves' (L) and two ranks of small 'leaves' (S). Two 'stipules' have glandular heads G, and two younger 'stipules' are marked by small arrowheads. Bar = 100 µm. (c) View of a node from the distal side, the shoot above removed, showing: the bases of large (L) and small (S) leaves; the three 'stipules' (St); a number of glands (G); and the site from which a bud has been removed (B). Bar = 200 µm. (d) Transverse section of a shoot taken just above the node. In addition to the same structures seen in Fig. 1c this photograph shows the attachment of 'glands' to the adaxial surface of the petioles (small arrowheads), the single large vascular bundle in each petiole, a small cross-connecting trace (arrow) almost fused with the large bundle in the petiole of the large 'leaf', and the vascular cylinder of the stem (in both bud and main axis). Bar = 200 µm. (e) A 'stipule' (St), with lateral gland (G), adjjacent to the lower edge of a large 'leaf'. The end of the stipule is also glandular (at arrow). Bar = 250 µm. (f) A gland (G) terminating a 'leaf' tooth. Bar = 100 µm. (g) Tuft of trichomes (T) at a 'leaf' tip. Bar = 100 µm.

The two 'leaf' traces are linked together by a small bundle. When the vascular system is followed upwards, this bundle branches off at approximately a right angle from the trace

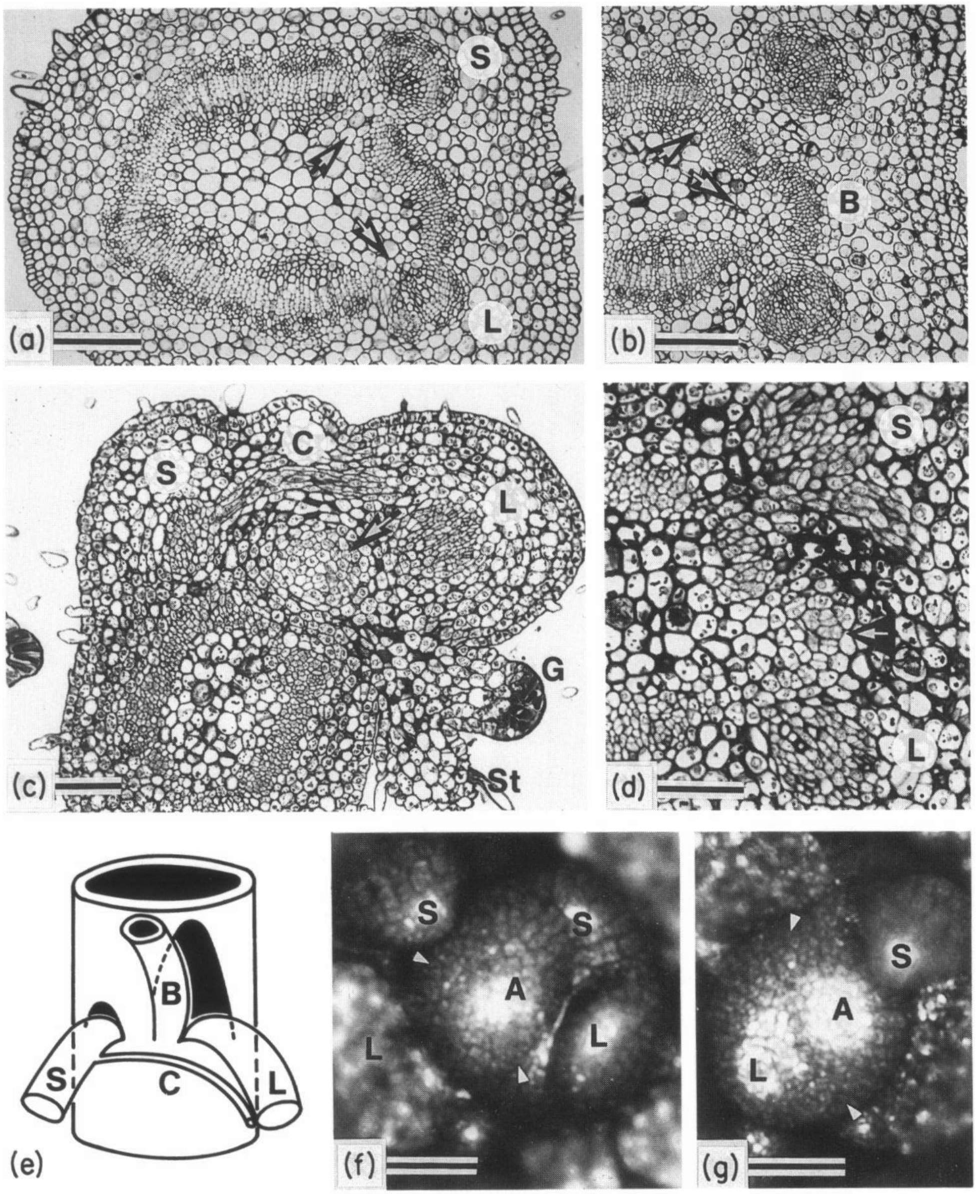

Fig. 2. (a and b) Transverse sections through a node. Bar = 100 μm. (a) Below node, showing cylindrical vascular system with leaf gaps (arrows) associated with the departure of the traces to the large (L) and small (S) 'leaves'. (b) Approximately 270 μm higher. The bud trace (B) departs from the leaf gap associated with the large 'leaf' (lower arrow). The upper arrow indicates the leaf gap associated with the small 'leaf', which has nearly closed at this level. (c) Transverse section through a young node, with the vasculature in the procambial state. The cross-connecting trace (C) is visible linking the traces (L) and (S) of the small and large 'leaves' respectively. The bud trace is indicated by an arrow. The bases of a 'stipule' (St) and 'gland' (G) are visible. Bar = 50 μm. (d) Transverse section through a rather younger node than that shown in (c). The traces to the large and small 'leaves' are indicated by S and L, and the developing bud trace is marked by an arrow. Bar = 50 μm. (e) Diagrammatic plan of the vascular system of a node. S and L are the traces to the large and small 'leaves' respectively, linked by the small cross-connecting strand C; B is the bud trace; leaf gaps are shown in black. (f) The earliest detectable stage in initiation of a primordium which will give rise to a pair of 'leaves', as an upgrowth (between the two arrowheads) of the shoot apex A. The next older pair of 'leaves' are labelled L and S on the right hand side of the apex, and an older (partly dissected) pair is similarly labelled towards the left. Bar = 50 μm. (g) A primordium which has grown upwards in the region L which will give rise to the large 'leaf', and has extended asymmetrically (extent delimited by arrowheads) around the apex A. S is the primordium of the small 'leaf' one node older. Bar = 50 μm.

of the small 'leaf' and runs toward the trace of the large leaf (Fig. 2c), then turns to run closely parallel with it (Fig. 1d). In young 'leaves' the small bundle is visible as a separate entity well up into the petiole, but by maturity further vascular differentiation makes the distinction hard to see.

The whole vascular plan of the node is shown in diagrammatic form in Fig. 2e.

Developmental morphology

The assorted organs at each node do not arise from separate primordia formed upon the shoot apex. The first sign of initiation of a new set of nodal structures is the appearance of a bulge in the apical meristem above the large 'leaf' two nodes down the stem (Fig. 2f). This is in the position which will be occupied by a large 'leaf'. The bulge becomes asymmetrically crescentic, extending laterally to include the region where the next small 'leaf' will be formed (Fig. 2g). A joint common 'leaf buttress' is thus formed, and this begins to resolve into individual primordia with the upgrowth of the dorsiventral primordium of the large 'leaf' (Fig. 3a). Then upgrowth of the similarly dorsiventral primordium of the small 'leaf' begins (Fig. 3b and c). By the time the primordium of the small leaf has become resonably distinct, the common 'leaf buttress' has also developed a small shoulder at the outer edge of the primordium of the large 'leaf' (Fig. 3b and c) and this finally gives rise to the radially symmetrical primordium of the 'stipule' at that edge (Fig. 3b and c). The two other radially symmetrical 'stipule' primordia arise later (Fig. 3d), that between the two 'leaves' arising before that at the outer edge of the small 'leaf'. The glandular tips of the 'stipules' become highly secretory early in development (Fig. 1b and 3e) so that even inner regions of the bud are sometimes partly covered by secretion. The 'glands' attached to the adaxial faces of the petioles develop much later (Fig. 3f).

As the 'leaves' develop, trichomes appear (Fig. 3d and e) and the marginal glands are initiated somewhat towards the adaxial face of the young leaf (Fig. 3g). Each small 'leaf' develops a conspicuous terminal gland (Fig. 3g), while large 'leaves' appear to develop only a terminal tuft of hairs (Fig. 3h). By the time the leaves are fully mature the terminal glands of the small 'leaves' have withered so that both types of 'leaf' only show an apical tuft of hairs, usually matted with secretion.

DISCUSSION

Singly inserted simple leaves with stipules are the normal state in the Flacourtiaceae (Warburg 1894; Hutchinson 1967) and indeed many other dicotyledons. The presence of stipules is commonly associated with a trilacunar nodal condition (i.e. the leaf is supplied by three vascular traces, each associated with a leaf gap in the stem vasculature) and this is the normal condition in the Flacourtiaceae (Sinnott & Bailey 1914). It is reasonable to assume that the exceptional condition in *Azara* is derived from this state. In the development of a leaf primordium with two stipules attached in the normal lateral position, a single primordium arises at first. This develops a wide insertion on the shoot apex and resolves later into a median leaf primordium and two lateral stipule primordia (e.g. in Vitaceae, Lacroix & Posluszny 1989). During the development of a nodal assembly in *A. microphylla*, the primordium arises at a single locus, extends laterally, and finally assumes an asymmetrically accentuated version of this 'normal' tripartite appearance. Up to this point the homology between the *Azara* leaf primordium and a 'normal' leaf primordium is maintained.

The majority of the subsequent divergences from the conventional may be explained by invoking well-known general processes, and this avenue is explored first. The small

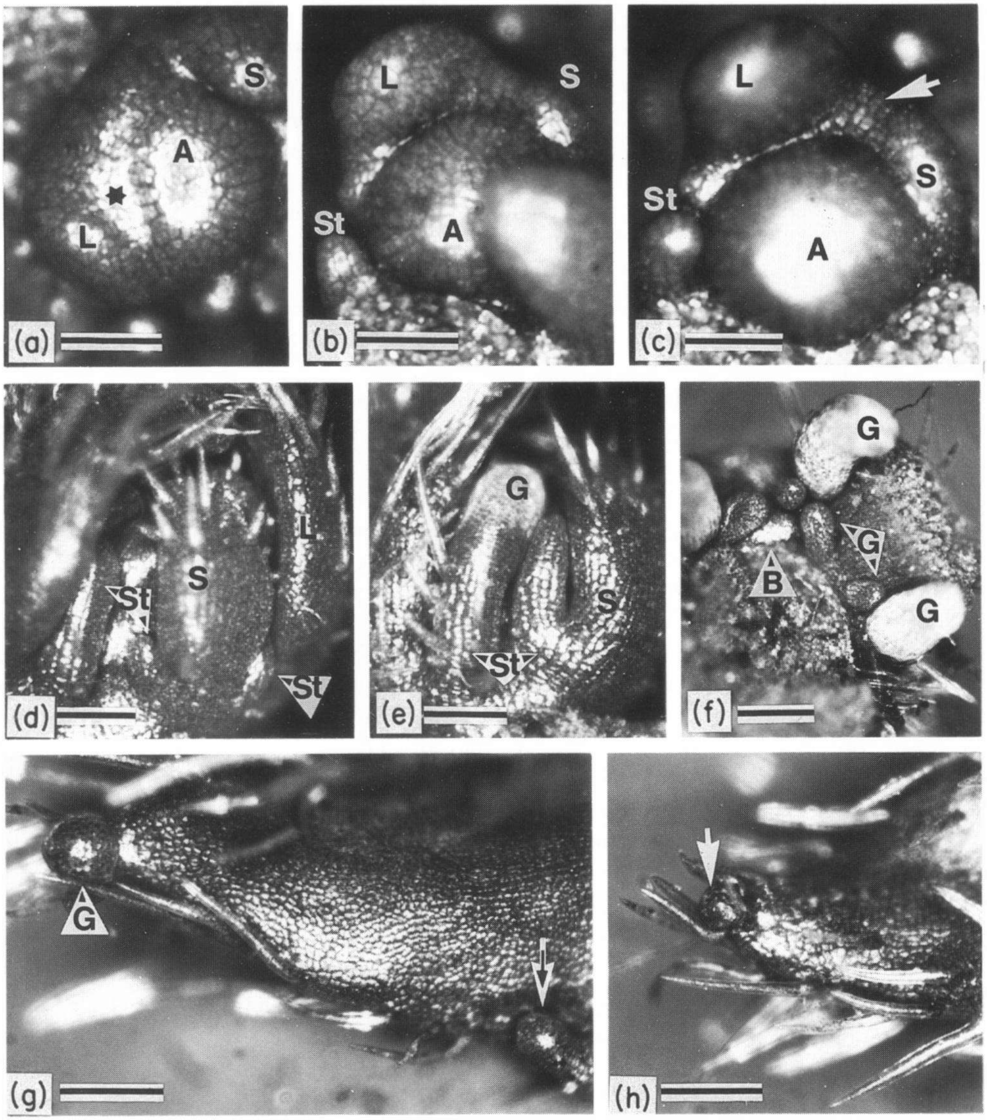

Fig. 3. (a) Common leaf buttress with the primordium of the large 'leaf' L. A triangular area (asterisk) may represent the future site of a bud. S is a small 'leaf' one node older. Bar = 50 µm. (b) and (c) Oblique views of the formation of the primordium of the small 'leaf' (S) beside the primordium of the large 'leaf' (L), the development of the primordium of the 'stipule' at the lower edge of the large 'leaf' at St, and the shoot apex (A). Also visible below the label St is the corresponding 'stipule' at the next older node, showing radial symmetry. Bar = 50 µm. (b) The primordium has developed two unequal shoulders. (c) Upgrowth of the small 'leaf' primordium is visible, and the 'stipule' primordium is beginning to appear. The 'stipule' between the two 'leaves' will arise at the arrow. (d) Lateral view of a partly dissected bud. Two young 'leaves' of one pair, labelled L and S, have developed some plain trichomes. At the left-hand label St are 'stipules' at the upper side of two small 'leaves', the smaller one belonging to the 'leaf' labelled S and the larger to the older leaf at the left. The right-hand label St indicates the 'stipule' between the two 'leaves' L and S. Bar = 75 µm. (e) Two 'stipules' St correspond to older stages of the two 'stipules' at the left-hand side of (d). The right-hand 'stipule' is associated with the small 'leaf' S. The left-hand stipule has developed a glandular tip G. Bar = 75 µm. (f) A partly dissected young node, seen from above. 'Glands', individually labelled G, are well developed; others, attached to the adaxial surface of the petiole and labelled by G in a white triangle, are much less mature. A young bud is visible at B. Bar = 100 µm. (g) and (h) Tips of the two young 'leaves' of a pair in which the large 'leaf' was 2·5 mm long and the small 'leaf' 1·5 mm long. Bar = 100 µm. (g) The small 'leaf' has a distinct gland G at the tip. The gland at the tip of a marginal tooth is indicated by an arrow. (h) There is no distinctive gland at the tip of the large 'leaf' (arrow), but there is a tuft of trichomes at this stage.

non-vascularized 'stipule' which develops from the small shoulder of the primordium adjacent to the outer edge of the large 'leaf' would be derived from a normal stipule by reduction, most particularly of the vascular supply. The radial symmetry is probably associated with reduction to little more than the stalk of a glandular structure. The large shoulder of the primordium, towards the upper side of the shoot, gives rise to the small 'leaf' in the position where another stipule would be expected. Replacement of this upper stipule by a 'leaf' would involve a process of homoeosis in the broad sense (Sattler 1988) or, more specifically, entropic homoeosis (Leavitt 1909) where a part of an organ is replaced by a structure resembling the whole original organ. Continuing this argument, the 'stipule' adjacent to the outer edge of the small 'leaf' at the upper side of the shoot becomes a rather normal stipule of the homoeotic leaf, reduced to a non-vascular structure like the 'stipule' at the lower edge of the large 'leaf', and the interpetiolar 'stipule' is shared between the two 'leaves' in the same way as interpetiolar stipules are shared between adjacent leaves in some Rubiaceae (Majumdar & Pal 1958; Rutishauser 1984). These 'stipules' are formed as part of the 'leaf' primordium as is normal for stipules, and this emphasizes the leaf-like nature of the homoeotic 'leaf' primordium as they arise in relation to it in much the same way that normal stipules arise with their associated leaf. This rationalization does, however, evade the rather significant question 'What controls the developmental relationship between leaf and stipule?'

In trilacunar nodes in general, the median trace runs directly into the leaf, and the lateral traces give off side branches which supply the stipules (Sinnott & Bailey 1914). The arrangement of the vascular system in *A. microphylla*, whereby each 'leaf' receives a separate trace from the stem vasculature, indicates how complete is the homoeotic replacement of stipule by 'leaf'. The proximal part of the leaf trace running into the small 'leaf', up to the point where the small cross-connecting trace diverges, presumably represents the proximal part of the lateral leaf trace; the cross-connecting trace represents the distal part of the lateral leaf trace; the distal part of the trace into the small 'leaf' represents stipule trace. The disproportionate development of the stipule trace would be induced by the homoeotic leaf primordium in the same way that a normal leaf primordium is generally considered to induce the formation of its own vascular connection (e.g. Steeves & Sussex 1989). The reverse view of the determination of vascular pattern, that the developing leaf trace determines the site at which a leaf primordium will appear (e.g. Larson 1975), could also readily be accomodated in an explanation based on homoeosis, i.e. a homoeotic conversion of a lateral leaf trace and stipule trace into a median or main leaf trace. Elimination of the other (lower) lateral leaf trace might be linked with the dorsiventrality of the shoot as in *Lotononis* (Dormer 1944) and perhaps also with the reduction of stipule development at that side.

It is perhaps surprising that there is only a single axillary bud at the node; in view of the fact that axillary buds appear to be induced by the axillant leaf (Snow & Snow 1942) one might expect each 'leaf' to subtend a bud. Probably the bud site is actually determined at an earlier stage of development (cf. Hussey 1971) when there is effectively only a single primordium. The connection of the bud vascular supply to the leaf gap of the large 'leaf' in any case probably reflects the original trilacunar condition of the node, as bud traces are commonly associated with the median leaf gap (Dormer 1972) and the asymmetrical attachment of the bud trace is related to the asymmetry of the subtending foliar structure.

Most of the unusual features of the shoot can be accommodated by the interpretation based on reduction of the stipule at the lower side of the large 'leaf', homoeotic replacement of the stipule at the opposite side by a 'leaf' so that the shoot becomes strongly dorsiventral, and insertion of further stipules in relation to the homoeotic 'leaf'. The position of the bud is not directly explained, but a fairly plausible hypothesis has been given. The interpretation is basically a typological one based on a 'classical' shoot model (cf. Rutishauser & Sattler 1985); other philosophically similar but less probable interpretations could be devised, for instance, one based on reduction and modification of a compound leaf. Even though the morphology of the shoot in *A. microphylla* is readily interpreted according to a 'classical' shoot model philosophically different interpretations can be reached which are complementary in the sense of Rutishauser & Sattler (1985). The homoeotic structure which replaces a stipule may be interpreted as a true leaf, receiving a leaf trace direct from the stem vasculature, or as a modified stipule. Similarly, complementary interpretations can be offered of the vascular supply of this organ, and its cross-connection with the large 'leaf'; even though the situation has apparently been derived from an unexceptional original relationship of lateral leaf trace and stipule trace.

In an investigation of the Rubiaceae, Rutishauser (1984) adopted, in addition to more classical models, a continuum approach which enabled him to postulate the existence of a range of structures intermediate between the classical categories of leaf and stipule. Similarly Rutishauser & Sattler (1986) proposed that in *Acacia longipedunculata* a morphological continuum could be considered to extend from trichomes through stipules to leaves (phyllodes). The continuum approach is useful where a morphological situation presents structures which do not fit neatly into categories with which the human brain is comfortable. However, it is not necessary to adopt this kind of approach to *Azara microphylla*, although a case could be made for the existence of a continuum between 'gland' and 'stipule'.

In *Acacia longipedunculata* annular girdling bulges or primordia, called whorl platforms (Rutishauser & Sattler 1986) arise on the shoot apex, and on each of these primordia are initiated the individual primordia of the stipules and phyllodes which make up a whorl. A similar phenomenon occurs in *Ceratophyllum* and *Equisetum* (Rutishauser & Sattler 1987) where girdling bulges formed on the shoot apex subsequently initiate the individual primordia of structures which subsequently appear to be leaves arranged in a whorl. In all these cases, and in *Azara*, the shoot apex appears to form common primordia on which a number of structures are initiated. However, in *Ceratophyllum* and *Equisetum* each common primordium gives rise to a number of similar structures. In contrast, in *Azara* and the case in *Acacia*, more than one kind of structure is formed. It was suggested (above) that homoeotic replacement of stipule by 'leaf' in *Azara* was accompanied by formation of 'stipules' in relation to the homoeotic 'leaf', and the situation in *Acacia verticillata* could be derived from the result of a similar process.

There remains in *A. microphylla* one detail which is a little difficult to rationalize. Why does the small 'leaf' develop a conspicuous terminal gland which is lacking in the large 'leaf'? As the 'stipules' have terminal glands, in this a relic of the hypothetical original stipule-like nature of the small 'leaf'? This curious aspect of detail actually need have no rational explanation in a direct morphological or functional sense. It may simply be a corollary of homoeosis. Ultimately, morphology is specified by genetic information in the present dogma. Developmental processes in plants are usually serial, e.g. the succession of parts in floral development, or serial and cyclic, e.g. the development of a shoot which can be seen as the production of a succession of modules each with their own serial pattern. At one extreme, interactions between components of developmental systems have been postulated at the level of control of gene expression, e.g. in the initiation of the succession of floral organs (Heslop-Harrison 1964). At the other extreme, there are examples of developmental control and correlation for which no control mechanisms have yet been suggested, e.g. the induction of axillary bud formation by the axillant leaf (Snow & Snow 1942). It is at least conceivable that the insertion of a homoeotic phenomenon of major proportions into the development of a leaf might interfere at some level with any of the developmental correlations which we see as 'normal'. So long as the results do not have a negative selective value, they could be perpetuated. This may be the explanation for the last minor eccentricity of *A. microphylla*, and perhaps other species of *Azara*.

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