A QUANTITATIVE STUDY OF ANISOPHYLLY IN ACER

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The fact that lateral or horizontal shoots often differ from vertical ones in showing a dorsiventral as opposed to a radial structure has long been observed. One aspect of this dorsiventrality in many species is the difference in size, and often in shape, between leaves on the upper and those on the lower sides of the branch. To this phenomenon the term "anisophylly" has been applied by Wiesner. The upper leaves are generally smaller than the lower ones, and those arising from the sides of the shoot are intermediate in size. There has been much discussion as to the factors which produce anisophylly—whether gravity, light, or various internal conditions are responsible—and the whole problem is of particular interest to students of morphogenesis in that it provides a fairly simple case for an analysis of some of the factors which determine form.

Wiesner (1868) and Frank (1868) were the first to consider the problem, and both believed anisophylly to be due primarily to gravity. In Picea and Acer, Frank twisted horizontal twigs, still attached to the plant, through an angle of 180° and tied them there. In Picea, the original anisophylly was maintained in the new growth, the leaves now on the upper side still being the longer, but the difference was not as marked as before. In Acer there was a complete reversal, the originally upper leaves, now on the lower side, being much longer than the originally lower ones. He found that anisophylly was not as marked in shoots grown in a dark chamber, thus suggesting that light might also play a part. Kny (1873) repeated Frank's experiment, using Abies instead, and found that the original anisophylly was finally reversed in the second season following the turning of the twigs. Frank (1873) also found that in shoots of Thuya there is a very definite anatomical difference between the upper and the lower sides. This difference was reversed when the twig was twisted through 180°; but as a result of shading experiments and on the basis of other evidence, Frank concluded that light, rather than gravity or an internal tendency toward bilaterality, was the factor involved. Goebel (1880) first suggested that internal factors were also at work in producing anisophylly. Rosenvinge (1889) showed that dorsiventrality may sometimes be due to external and sometimes to internal factors.

Wiesner in his later papers emphasizes more strongly the importance of light. At first he regarded the large size and long petioles of leaves on the lower sides of anisophyllous shoots as due to etiolation. Later (1894), he concluded that these leaves actually got more light and thus grew larger than the upper ones. He also noticed that it was always the leaf on the abaxial side of the twig which grew larger, and for this general tendency for structures on the outside of lateral shoots to become larger than those on the inside, next the mother axis, he proposed the term "exotrophy" (1892). He believed this to be due to the better nutrition of these outwardly placed structures, and suggested that exotrophy might be an important factor in anisophylly. Weisse (1895), working with Acer, showed by shading experiments that the leaves which were shaded were smaller than those in the light. He also put young maples on a horizontal clinostat, thus eliminating the effect of gravity, and found that, although anisophylly persisted somewhat, it was less marked than in the control plants. Weisse stresses gravity rather than light as a factor in anisophylly. He agrees with Wiesner that exotrophy is also concerned, but does not believe that nutrition is the cause of it. He renamed the phenomenon "ectauxesis," and believed it to be due to inherited morphological factors and perhaps to be teleological. Figdor (1897) caused the side shoots of eight species to grow vertically; later, he (1904) planted a young maple tree in such a position that one of the side branches was vertical, and in this position these lateral shoots ultimately lost their anisophylly. Vertical shoots bent downward often acquired it. Figdor believes that gravity, light, and possibly certain internal factors are at work. Nordhausen (1902) regards light as an important factor in leaf asymmetry and anisophylly in Aesculus, where those portions of the leaf-blade presumably exposed to greater light are thicker than the rest; but holds that light plays but a small part in the case of Acer. He regards exotrophy as an important factor. Heinricher (1910) concludes that differences in transpiration rate, governed by temperature, are important in producing anisophylly in Sempervivum. This is denied by Doposcheg-Uhlár (1913), who attributes conditions in this genus to light and gravity only. Boshart (1911) believes that leaf asymmetry (and presumably other phenomena of anisophylly) are controlled chiefly by conditions at the growing point, such as the space occupied by the leaf primordia and their relation to the rest of the meristem. A survey of the literature up to 1909 is presented by Figdor (1909).

A number of factors may evidently be concerned with the production of anisophylly, but there is no unanimity of opinion as to the part played by each. The purpose of this paper is to present results of a statistical study of leaf measurements on anisophyllous twigs of Acer, the genus which has been most frequently studied in this connection, for the purpose of reducing the problem to a quantitative basis and in the hope that a knowledge of the relative dimensions, shape, and variability of leaves occupying different positions on the lateral shoots may produce evidence of value.

Maple twigs growing horizontally show marked anisophylly. The upper member of each vertically oriented pair is distinctly smaller than the lower and differs from it somewhat in shape. The members of the horizontally oriented pair are intermediate in most characters between the upper and the lower members of the vertical pair. They are also asymmetrical, the lower one of the two main lateral veins being longer than the upper.

1000 leaves of *Acer saccharum*, growing on twigs which were horizontal or essentially so, were studied. In order to avoid possible genetic differences, they were all taken from a single tree of this species. Only those twigs were chosen in which the successive leaf pairs were clearly vertical and horizontal, all those in which the pairs were at all oblique in their insertion being eliminated. Twigs were chosen from all parts of the crown of the tree. All the leaves on each twig were recorded, so that there is an equal representation of those from the basal, median, and terminal regions of the year's growth. Leaves were harvested in midsummer after complete maturity had been reached. Length of petiole, length of midrib, width of blade from tip to tip of the lateral lobes, and length of right and left main

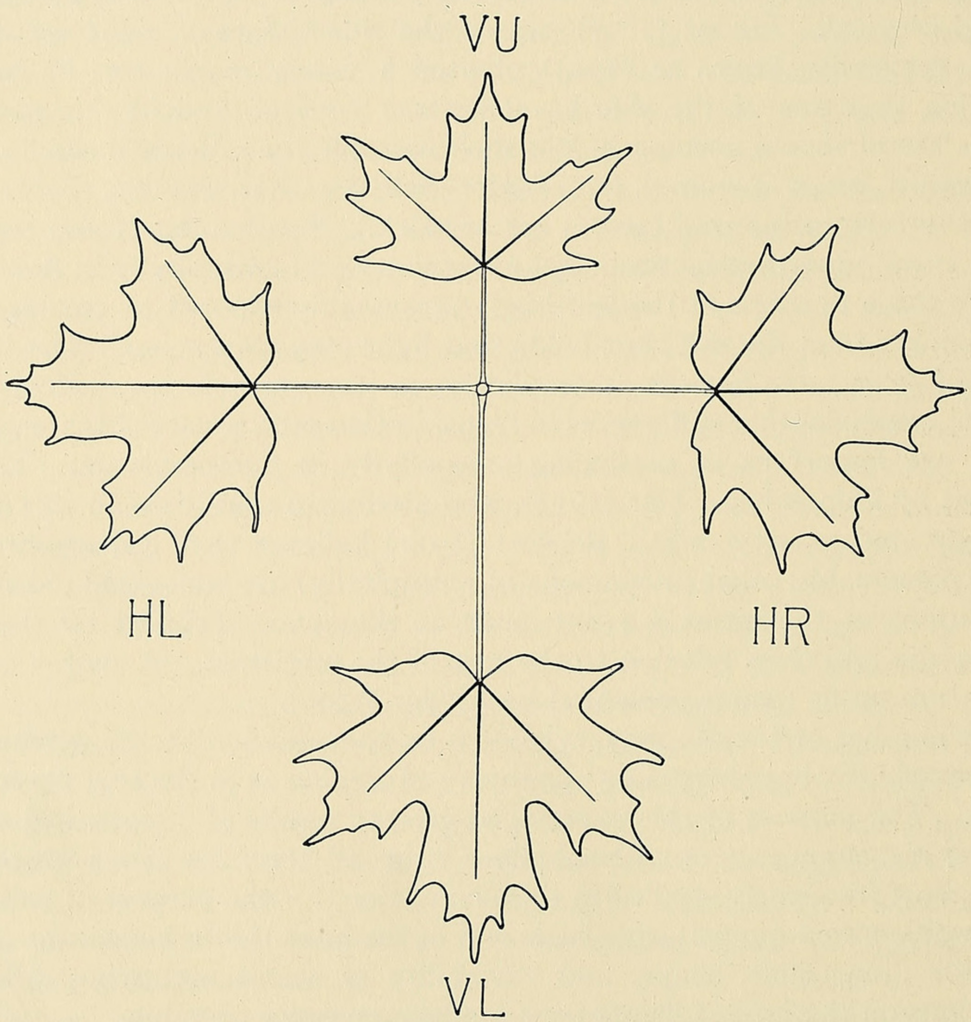

Fig. 1. Diagram representing the four groups of leaves, dimensions proportional to the means in table 1. VU, vertical upper leaf; VL, vertical lower; HR, horizontal right; HL, horizontal left.

Table I. *Mean, Standard Deviation, and Coefficient of Variability for the Various Size Characters in the Total Population and in the Four Leaf Groups*

	M.	σ	C.V.	Number
Length of Midrib (cm.)				
Total	*9.50 ± .059*	*2.75 ± .041*	*29.0 ± .508*	*1000*
Vertical upper	7.72 ± .102	2.44 ± .075	31.6 ± 1.106	258
Vertical lower	10.59 ± .114	2.73 ± .081	25.7 ± .858	258
Horizontal right	9.86 ± .103	2.37 ± .072	24.0 ± .815	242
Horizontal left	9.89 ± .108	2.48 ± .076	25.0 ± .858	242
Length of Right Rib (cm.)				
Total	*8.19 ± .055*	*2.79 ± .042*	*34.1 ± .620*	*1000*
Vertical upper	6.85 ± .102	2.43 ± .072	35.6 ± 1.301	258
Vertical lower	9.07 ± .123	2.94 ± .087	32.4 ± 1.145	258
Horizontal right	8.85 ± .117	2.70 ± .083	30.5 ± 1.094	242
Horizontal left	7.98 ± .109	2.51 ± .077	31.5 ± 1.140	242
Length of Left Rib (cm.)				
Total	*8.23 ± .060*	*2.81 ± .042*	*34.2 ± .621*	*1000*
Vertical upper	6.86 ± .101	2.41 ± .071	35.1 ± 1.271	258
Vertical lower	9.12 ± .124	2.97 ± .088	32.5 ± 1.150	258
Horizontal right	8.06 ± .108	2.49 ± .076	30.9 ± 1.116	242
Horizontal left	8.80 ± .118	2.72 ± .084	30.9 ± 1.116	242
Width of Blade (cm.)				
Total	*12.86 ± .084*	*3.94 ± .059*	*30.6 ± .539*	*1000*
Vertical upper	11.18 ± .153	3.65 ± .108	32.7 ± 1.165	258
Vertical lower	13.72 ± .177	4.22 ± .126	30.7 ± 1.067	258
Horizontal right	13.31 ± .159	3.65 ± .112	27.4 ± .957	242
Horizontal left	13.19 ± .161	3.71 ± .114	28.1 ± .989	242
Thickness of Blade (mm.)				
Total	*.1607 ± .0004*	*.0201 ± .0003*	*12.5 ± .196*	*1000*
Vertical upper	.1609 ± .0008	.0200 ± .0006	12.4 ± .380	258
Vertical lower	.1608 ± .0008	.0195 ± .0006	12.1 ± .369	258
Horizontal right	.1603 ± .0008	.0190 ± .0006	11.8 ± .373	242
Horizontal left	.1614 ± .0009	.0202 ± .0006	12.5 ± .394	242
Area of Blade (sq. cm.)				
Total	*64.84 ± .734*	*36.13 ± .528*	*55.7 ± 1.257*	*1008*
Vertical upper	47.60 ± 1.090	25.99 ± .770	54.6 ± 2.429	258
Vertical lower	78.90 ± 1.713	40.81 ± 1.213	51.7 ± 2.210	258
Horizontal right	70.20 ± 1.485	34.30 ± 1.050	48.9 ± 2.099	242
Horizontal left	68.40 ± 1.448	33.20 ± 1.016	48.5 ± 2.068	242
Volume of Blade (cc.)				
Total	*1.049 ± .0127*	*.594 ± .0089*	*56.6 ± 1.281*	*1000*
Vertical upper	.757 ± .0189	.439 ± .0135	58.0 ± 2.648	258
Vertical lower	1.242 ± .0280	.668 ± .0198	53.8 ± 2.352	258
Horizontal right	1.112 ± .0239	.553 ± .0168	49.7 ± 2.145	242
Horizontal left	1.071 ± .0241	.555 ± .0170	51.8 ± 2.291	242
Length of Petiole (cm.)				
Total	*8.35 ± .099*	*4.64 ± .070*	*55.5 ± 1.245*	*1000*
Vertical upper	4.58 ± .093	2.21 ± .066	48.2 ± 1.988	258
Vertical lower	11.17 ± .213	5.07 ± .151	45.4 ± 1.823	258
Horizontal right	8.85 ± .174	4.00 ± .123	45.2 ± 1.816	242
Horizontal left	8.89 ± .177	4.08 ± .125	45.9 ± 1.918	242
Diameter of Petiole (mm.)				
Total	*1.509 ± .0091*	*.425 ± .0064*	*28.2 ± .489*	*1000*
Vertical upper	1.236 ± .0142	.337 ± .0100	27.2 ± .920	258
Vertical lower	1.679 ± .0187	.437 ± .0130	26.0 ± .873	258
Horizontal right	1.569 ± .0174	.401 ± .0123	25.6 ± .883	242
Horizontal left	1.572 ± .0164	.378 ± .0116	24.0 ± .819	242

lateral veins (as seen from the upper or ventral leaf surface) were recorded. Petiole diameter was measured at the mid-region of the petiole by a micrometer caliper. Blade thickness was also measured by a micrometer caliper at two symmetrically situated points at right and left of the midrib in a region where there were no projecting veins and where mesophyll thickness alone could be determined. These two measurements were almost always identical, but when different were averaged. Blade area was determined by outlining the blade on standard-weight paper, cutting this out, and weighing the cut-outs. Blade volume was calculated by multiplying area by thickness.

Constants were determined for the whole population and for each of the four groups into which it was divided: vertical upper and vertical lower—the upper and lower members of the vertically oriented leaf pairs; and horizontal right and horizontal left—the right and left members of the horizontally oriented pairs as seen by an observer facing the apex of the twig (fig. 1). These constants are set forth in table 1.

An inspection of this table brings out certain facts as to the differences between these leaf groups in *dimensions*, *shape*, and *variability*.

Differences in Dimensions

A comparative study of the means shows that in all measurements (except blade thickness) the vertical upper leaves are markedly smaller than the lower ones, and that the members of the horizontal pairs are in all cases intermediate between these two. A significant fact which the figures bring out is that the combined size of the two members of the vertical pairs approximately equals the combined size of the two members of the horizontal pairs. In other words, the plant has a given amount of leaf material available at a given node (of course this amount decreases as we approach the apex of the twig), but this material is divided between the two leaves at this node in varying proportions, depending on the orientation of the particular leaf pair which arises there.

Perhaps the most striking fact brought out is that the four leaf groups are of almost exactly the same blade thickness. Whatever are the factors which produce the differences between these groups in other dimensions, they evidently do not affect this one. This uniformity cannot be due to lack of delicacy in measurement, for decided differences in thickness were recorded between different leaves, the readings running from 0.12 to 0.25 mm. Leaves on the same twig all tended to have the same thickness, but twigs differed among themselves, those from relatively shaded positions in the tree tending to be thinner than those which were more exposed to the sunlight. If light intensity determines blade thickness, as commonly supposed, we must infer that on a given twig the leaves in these four positions receive approximately the same degree of illumination.

Differences in Shape

The leaf groups also differ in shape. It will be noted that the members of the vertical pairs are symmetrical, the right and left main veins being equal. In the horizontal leaves, however, the lower or abaxial main lateral veins (right vein of the right leaf and left vein of the left) are distinctly longer than the upper ones, this difference being more than five times its probable error.

In the ratio of petiole length to blade (midrib) length the four groups also differ, as is shown in table 2. In the vertical lower leaves the petiole is far longer in proportion to the blade than it is in the vertical upper; and the members of the horizontal pairs are again intermediate.

Table 2. *Ratio of Blade Length to Petiole Length in the Four Leaf Groups*

	Blade Length / Petiole Length
Vertical upper	1.69
Vertical lower	.95
Horizontal right	1.11
Horizontal left	1.11

Finally, the four groups differ in blade shape, as shown by the width-to-length ratio in table 3. The vertical upper leaves are relatively short and broad, the vertical lower ones relatively long and narrow, and the horizontals intermediate.

Table 3. *Ratio of Blade Width to Blade Length in the Four Leaf Groups*

	Blade Width / Blade Length
Vertical upper	1.45
Vertical lower	1.29
Horizontal right	1.35
Horizontal left	1.33

Differences in Variability

A study of table 1 shows that some dimensions are much more variable than others,[1] petiole length having the highest coefficient of variability and blade thickness the lowest. For any given size character the four groups also differ in variability in many cases. In every instance (except blade thickness), the vertical upper leaves are much more variable than the horizontal ones, and the vertical lower are usually intermediate. Although the difference between the vertical upper and the horizontal leaves is not in every case significant in comparison with its probable error, the figures justify us in regarding the vertical upper leaves as the most variable of the four groups.

[1] The coefficients of variability for those size characters which involve two dimensions (blade area) or three dimensions (blade volume) are of course necessarily higher than, and thus not comparable with, those involving only one dimension.

Another difference in variability which is noteworthy is that between the length of the midrib and of the lateral ribs, especially in the horizontally oriented leaves. Here there is an average difference of 6.4 percent, which is more than four and a half times its probable error and thus evidently significant.

Other Evidence

Aside from these gross measurements, the authors examined the terminal buds of horizontal shoots during the winter and found (as has been noted by others) that the primordia of the four leaf groups were essentially equal in size, indicating that visible differentiation, at least, does not begin until the shoot starts to grow. The experiments of earlier workers on twig-reversal were also repeated and confirmed, it being found possible to reverse the anisophylly completely by twisting a horizontal stem through an angle of 180°, provided this was done before the buds opened.

Discussion

We should now consider the bearing of our results on the various theories which have been put forward to account for anisophylly.

The evidence with regard to light is significant. It is a matter of common observation that the thickness of the blade is generally directly proportional to the amount of light it receives during development. The fact that the four leaf groups are identical in blade thickness therefore justifies us in concluding that they must receive essentially the same amount of sunlight and that light cannot be a factor of very great importance in determining the marked differences which exist between them.

The case is different with regard to gravity, however. Not only are all the foliar structures on the lower half of the branch much larger than on the upper, but the shape of these organs is what we should expect to result if the downward pull of gravity is really effective in development; for the vertical upper leaves are "telescoped," the blade being relatively short in proportion to its width and the petiole relatively short in proportion to the blade; and the vertical lower leaves are extended or drawn out, the blade being relatively long in proportion to its width and the petiole relatively long in proportion to the blade.

It may be objected that these results are really due to some other factor and that neither these facts nor the experimental data gathered by ourselves and others at all prove that gravity is really the dominant factor concerned. In this connection the results as to relative variability are of interest. We have shown that the vertical upper leaves are the most variable of all in their size characters (the vertical lower ones probably ranking next), and that the lateral veins in the horizontal leaves are more variable in length than the midrib. This is difficult to explain until we study individual twigs and find that the intensity of the anisophylly which they display is not uniform but varies somewhat from twig to twig. Furthermore, an examination of twigs growing in all orientations shows that anisophylly appears very slightly in those twigs which are but slightly inclined from the vertical and becomes more and more marked as we approach the horizontal. The upper leaf is therefore relatively smaller in proportion to the lower in some of our twigs than in others; and since we have shown that the amount of leaf material at a given node tends to be independent of the way in which this material is apportioned between the two leaves, it follows that these differences in relative size between upper and lower leaves will be much greater *in proportion to the size of the leaf* in the upper leaves than they are in the lower, and that the variability in size of the upper leaves will therefore be greater than that of the lower, the result which we actually find to exist. The horizontal leaves, being relatively unaffected as to their volume, area, petiole length, and midrib length by these slight differences, will tend to be the least variable of all. In these leaves, however, the difference in length between the upper and lower main lateral veins also varies with the degree of anisophylly and thus with the orientation of the twig, increasing as we approach the horizontal. In a group of twigs varying slightly in the degree of anisophylly which they show (as do ours), these lateral veins will therefore tend to be more variable in length than the midrib, as we found actually to be the case. These differences in degree of variability to which we have called attention are probably only the natural result of slight differences in the intensity of the anisophylly displayed, which in turn seem to be due to, or at least to parallel, differences in *the orientation of the twigs to the horizontal.* Although this does not prove that gravity is the major factor here operating, it certainly favors such a conclusion.

As to exotrophy and other internal factors which involve a relation between the twig and its mother-shoot and are believed by Wiesner and others to stimulate growth on the outer (thus in horizontal twigs the lower) side of the stem, our evidence is not of decisive value. Such a factor may be operative but it is evidently not constant in its effect, since we find that twigs vary in the degree of their anisophylly. It may be argued that the size of the angle between twig and mother-shoot may determine the intensity of anisophyllous development, but a study of individual branches does not confirm such a conclusion but rather suggests that absolute orientation to the horizontal is the chief factor.

Evidence from this biometrical study of anisophylly therefore suggests that gravity is the major factor in producing the phenomenon in Acer. Other factors, both external and internal, may perhaps play a part, and the only conclusive evidence as to their relative importance must be derived from carefully controlled experimental work.

Summary

1. In horizontal twigs of certain species there are marked differences between the leaves borne on the upper and those on the lower sides of the twig. Difference of opinion exists as to the causes of this anisophylly.

2. In horizontal anisophyllous twigs of a single tree of *Acer saccharum*, measurements were made of the various linear dimensions and of blade area and volume in 1000 leaves, these being divided into four groups according to their position on the twig—the upper and lower members of the vertically oriented pairs and the right and left members of the horizontally oriented pairs.

3. The vertical upper leaves are the smallest, the vertical lower the largest, and the horizontal leaves are intermediate between these. At a given node the combined size of the two members of a leaf pair is essentially the same regardless of whether the pair is a vertically or a horizontally oriented one.

4. In thickness of leaf-blade, the four groups are almost identical.

5. In proportion to their midrib length, the vertical upper leaves are the broadest and have the shortest petioles; the vertical lower leaves are the narrowest and have the longest petioles, and the two horizontal leaves are alike and intermediate between the two former. In each horizontal leaf the lower lateral vein is markedly longer than the upper.

6. The vertical upper leaves tend to be the most variable in their dimensions, the vertical lower next, and the horizontal leaves the least. In the horizontal leaves the two main lateral veins are more variable than the midrib. These differences in variability are evidently due to the fact that the twigs studied differ somewhat in the degree of anisophylly which they display. This is probably due, in turn, to slight differences in the orientation of the twigs with reference to the horizontal, since a study of the degree of anisophylly in this species indicates that its intensity is proportional to the degree to which the twig diverges from the vertical.

7. Evidence here presented indicates that gravity is more important than light or other environmental or internal factors in producing anisophylly in Acer.

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