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EXPERIMENTS ON BIJUGATE APICES

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The bijugate phyllotaxis and the shoot apices of seedlings of *Dipsacus laciniatus* are briefly described and discussed. The normal mean angle between successive pairs of leaves was found to be 72°·7.

Experiments are reported which were designed to test a suggestion that in bijugates the deviation of each new pair from a position at right angles to that of the previous pair is due to an inhibition from the second older pair below. The leaves of the apical buds were removed as far as the youngest pair (the P_1's) inclusive, or as far as the P_2's or P_3's only. After the operations the angles between successive pairs increased considerably, and the increases continued for five or even six plastochrons. Also some angles increased beyond 90° and reversed the spirals. It is pointed out that these results are difficult to reconcile with the inhibition hypothesis.

Comparison is made with the bijugy of apices of *Salvia horminoides*, which can be explained junctionally as due to the contacts 1, 1 and 2 of the leaf bases.

For the bijugy of *Dipsacus* an explanation is suggested in terms of the combined working of two factors. The first factor is junctional and depends on the shapes of the leaf bases with their united rims. The second factor is a pressure exerted by the upstanding parts of the P_2's on the sides of the stem apex, which they were seen to touch. The results of the experiments can also be understood on this basis.

1. Introduction

In the simplest bijugate phyllotaxis it can readily be seen in bud sections that the young leaves are arranged in pairs of opposite members, just as in the common decussate phyllotaxis, but that the plane of each pair is not at right angles to that of the previous pair. For each of these planes deviates a little from the right angle, often from 10 to 20° (figure 1), and these deviations are all in the same direction in any one bud, though not in different buds of the same plant. Thus the arrangement is both paired and also spiral. It is therefore not possible to draw one genetic spiral through all the leaves in their order of formation, as it is in simple spirals, but one can draw through them two parallel genetic spirals, of which each one passes through one leaf of each pair. This simple bijugate phyllotaxis is regular and characteristic in the rosette stages of the Dipsacaceae, and is found in the rosettes of a few species of other families (see figure 17, and Church 1904, p. 167). It undoubtedly exposes the leaves of these rosettes to light much better than would straight decussation, which is rare in rosette stages and seems very unsuitable for them. In the rosettes of *Dipsacus* each new pair of leaves makes contact below it with the next older pair only.

Later in the growth of the Dipsacaceae, when the buds have elongated and begin to form the inflorescences, the arc covered by each lateral member diminishes, new contacts are formed, the phyllotaxis rises and parastichies become conspicuous. The numbers of these parastichies, or spirals of two sets passing through the lateral members and winding round the apex or axis in opposite directions, are commonly twice those successive numbers of the Fibonacci series which are found in simple spirals (see Church 1904, chapter 17 and illustrations), and the divergence angle along each of the genetic spirals (as distinct from the steeper parastichies) comes very close to 68°·5, or half the Fibonacci angle of 137°, as was pointed out by the Bravais (1837). This can be verified on figure 74 of Church (1904). The lateral members are still paired, but the pairs are less conspicuous.

The way in which these higher bijugate systems are developed from simple bijugy as the arcs diminish and the contacts change seems to present no *special* problem, but to be quite similar to the ways in which simple Fibonacci spirals are developed from distichy and from straight decussation, of which van Iterson (1907) has given much the best account. But the simple bijugy itself does present a special problem, and the present paper will be concerned with an attempt to discover its causes.

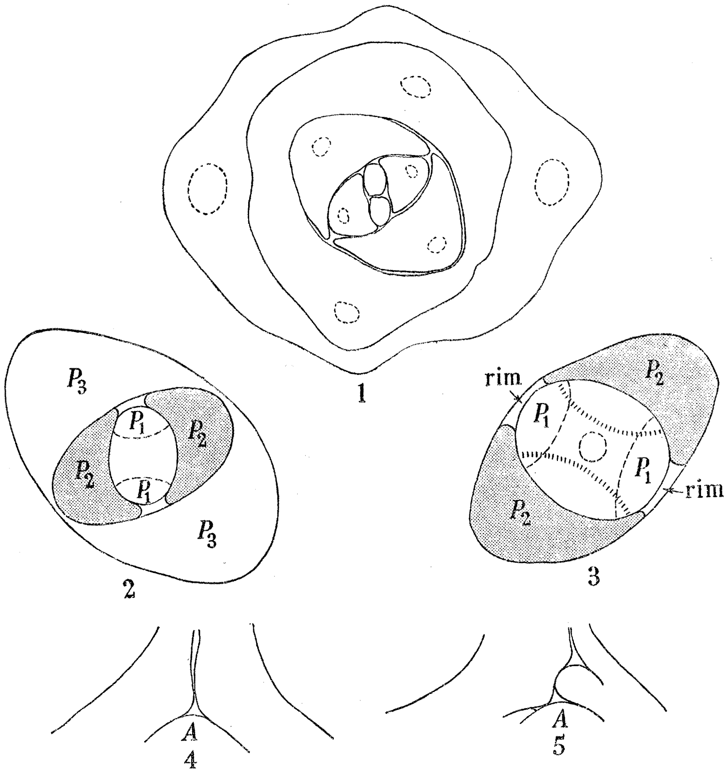


FIGURE 1. *Dipsacus laciniatus*. Cross-section of normal bud (magn. × 17).

FIGURES 2, 3. Normal apices exposed, diagrammatic. The P_2's and P_3's have been cut down.

FIGURES 4, 5. Normal apices in longitudinal section (magn. × 40). Stem apex is marked *A*.

Church (1904) has indeed tried to apply to bijugy his 'parastichy' theory, according to which the leaves are determined at intersections of the parastichies of the two opposite sets. For this purpose he points out that through the leaves of a rosette of *Dipsacus* one can draw two opposite sets of spirals, which he calls the parastichies, one set containing two spirals and the other four; and that if the numbers of both sets are even, then the phyllotaxis must be bijugate. But a weakness of this interpretation is that along the spirals of the set of four the bases of the youngest leaves are not in contact, in *Dipsacus* at least, as the writer finds, so that it seems arbitrary to select these spirals and consider them causally significant. (Church's drawings, figures 62*a*, *b*, do not show the contacts of the youngest leaves.) In any case Church's whole parastichy theory (1904) can hardly be considered to be a clearly worked-out causal theory, whatever may be its value as a system of geometric constructions imitating phyllotaxis. Church has, however, contributed many valuable observations and drawings of the higher bijugate systems of the inflorescences of Dipsacaceae.

It is also difficult to explain the simple bijugy of rosettes of Dipsacaceae on the basis of any junctional theory of phyllotaxis, if the term 'junctional' introduced by Schoute (1913) may be used for any theory according to which the positions of new lateral members depend on the existing members directly neighbouring to them. For in *Dipsacus* at least, as the writer finds, each new leaf makes contact with the members of the next older pair only, so that on junctional grounds a straight decussation would be expected unless there were some special complicating factor. Recently, however, Richards (1948, p. 231) has made a suggestion to explain simple bijugy which goes beyond a purely junctional theory. He supposes that the positions of any new pair of leaves n are modified by inhibitions or repulsions from the second older pair $n-2$ below them, although the leaves n never make contact with that pair, but are separated from it by the flanks of pair $n-1$ (see figure 2). These inhibitions cause the plane of pair n to deviate a little from the plane at right angles to $n-1$, and when once these deviations are started they will clearly continue in the same direction. Such a process would be important, if genuine, and the suggestion attracted the writer very much, especially since for several years evidence had been accumulating that in *Rhoeo discolor*, a monocotyledon with spiro-distichous phyllotaxis, the new leaves are indeed repelled in a similar way by older leaves with which they do not make contact (Mary Snow, 1951); and spiro-distichy is related in form to straight distichy rather as simple bijugy or spiro-decussation is to straight decussation.

In *Lupinus albus*, indeed, we concluded that the position of a new leaf is not appreciably affected by any other existing leaves except those with which it makes contact (Snow, M. and R. 1931, pp. 17, 27; 1933, p. 377; 1947, p. 15). But it seemed possible that in the Dipsacaceae, with their peculiar phyllotaxis, a different factor might be at work, as it is in *Rhoeo*. Accordingly the writer has experimented by operating on shoot apices of *Dipsacus laciniatus*, and these experiments and various observations will here be reported and discussed.

2. THE NORMAL APICES AND PHYLLOTAXIS OF *DIPSACUS LACINIATUS*

Since the leaves of each pair are fairly accurately opposite, the divergence angles were taken to be the angles between the median planes of successive pairs, which are simpler and easier to measure than the separate angles along the two genetic spirals (see figure 1). Thirty angles were measured, in sections of apical buds of eight plants pickled at about the same stage as at the end of one of the experiments to be reported. The mean angle was 72°·7. The standard deviation was 3°·61, and the standard deviation of the mean was 0°·66. The extremes were 68° and 80°. The apical part of the axis, apart from the actual stem apex or apical cone, is slightly crater-shaped, so that even the older leaves of the bud are cut close to their bases in cross-sections just above the stem apex, and show the divergence angles accurately.

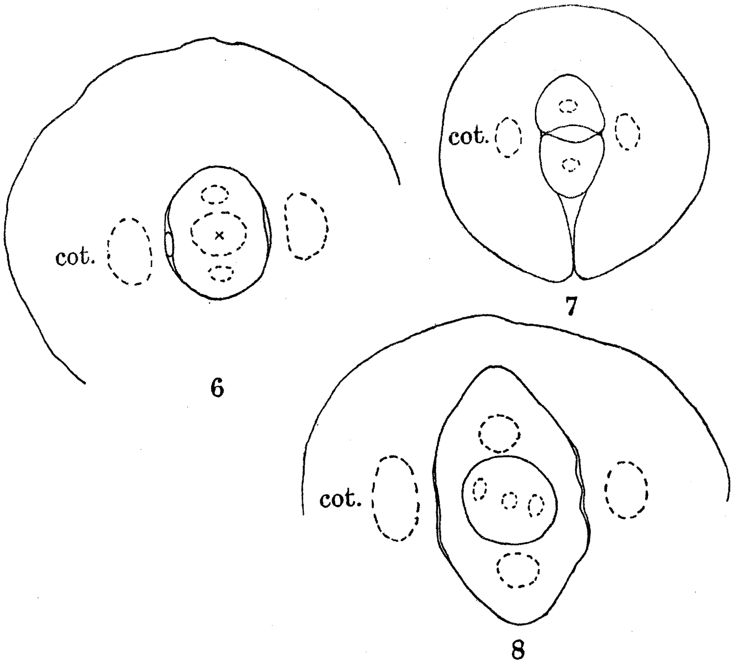
On the other hand, the sections through the stem apex did not show clearly the shapes and relations of the stem apex and youngest leaves. For the apical cone and the youngest pair of leaves, or their bases, appeared only indistinctly, rising up from a nearly flat disk of tissue which formed the background, and the next older pair were often inserted at a higher level and did not appear in the same section. More instructive therefore were observations of this region in the solid made with a Leitz Greenough binocular at magnification × 50 or × 75 giving stereoscopic effect; and observations of this kind were made whenever the P_1's or young P_2's were removed in an experiment. (The P_1's and P_2's are the youngest and second youngest pairs of leaves visible at the time of operation, and the pairs arising after the operations will be called the I_1's, I_2's and so on, as before.)

In some ways the apices are similar to those of decussate species, except of course that the divergence angle is not 90° (see figure 2). For each new pair of leaves arises from the narrow ends of a stem apex which seen from above near the end of a plastochron appears elliptic, except that the narrow ends often look a little flattened: and after this the remaining stem apex is extended transversely to form an ellipse in the other direction. Also the youngest pair makes contact at its base with the next older pair only, as in decussates. But in *Dipsacus* a striking point is that the leaves of the youngest pair unite by low rims at the end of the plastochron, as was noticed several times. These rims can be seen in figure 3, which, like figure 2, shows the appearance of an apex in which the P_2's and older leaves have been cut down. The shaded areas represent the cut surfaces of the P_2's, and the P_1's are just arising. The P_2's are here the youngest pair with united rims, since the plastochron is at an early stage.

Another point of interest is that the upper parts of the youngest leaf-pair but one (the P_2's in the experiments) bulge forward between the youngest pair, and at about mid-plastochron they touch the apical cone or stem apex over a considerable area, though they do not quite touch its extreme summit. These contacts are illustrated in figures 4 and 5, as seen in longitudinal sections. Also observations of apices in the solid often revealed this process, and showed that the P_2's already touch the stem apex at the beginning of the plastochron. Indeed in one plant a pair of old P_1's was found to be already touching the apex. The contact of the P_2's with the apex reaches closest to its extreme summit at about mid-plastochron, and later the bulging part of the P_2's is carried up away from the apex, and the P_1's curve forward and meet beneath it at their tips. Further, when a pair of P_2's which touched the apical cone was removed by being pressed outwards, as will be described, they sometimes left a nearly transparent film of some kind adhering to the apical cone (figure 3), so that these leaves must actually have been pressing on the apical cone and adhering to it slightly. These films were of course quite distinct from the remaining bases of the leaves removed, and their upper boundaries, towards the summit of the apical cone, were seen as brown lines, and showed how far up the apical cone the contact of the P_2's had reached. The brown lines are represented in figure 3 by the lines of dashes drawn crosswise: it can be seen that they reach up the stem apex almost to the little apical dome which is conspicuous when the plastochron is at an early stage, as it was in this observation. These contacts of leaves with the stem apex are unusual, and may be characteristic of Dipsacaceae. The significance of these contacts and of the united rims will be discussed in §5.

The start of the bijugate phyllotaxis in seedlings is also of interest. In sections of fourteen seedlings, only just germinated, the first divergence angles, those between the cotyledons and the first leaf pair, were found to be very close to 90°, having a mean value of 86°·6 (see figures 6, 7, 8). The lowest two were 83° and three were exactly 90°. But the second divergence angles were found in eight young seedlings to have a mean value of 71°·1 (extremes 62 and 84°). With the second angle the deviation of the phyllotaxis is established, and it then continues in the same direction. But the small deviations of the first angle from 90° must be accidental only, since they were found to be often in the direction opposite to that of the second and subsequent angles, as in figure 8.

These points need to be observed in bud sections: for in the intact seedlings it looks as if the first divergence angle regularly deviates from 90° considerably and in the same sense as the subsequent angles. But this is apparently because the blades of the first pair of leaves shift by lateral curvatures from under those of the second pair. Thus in two of the above seedlings in which the first angles were seen in sections to be 89° left and 90°, they had both been estimated on the intact seedlings as 70° right.



FIGURES 6–8. Bud sections of very young seedlings (magn. 6, ×47; 7, 8, ×40).

In the axillary buds the development of the bijugate phyllotaxis is difficult to follow, since they are very much flattened when young, apparently by pressure from the leaves above and below them, and their phyllotaxis is thereby made irregular and abnormal.

3. THE NATURE OF THE EXPERIMENTS AND THE METHODS

In order to test the suggestion of Richards, the developing leaves of rosettes of *D. laciniatus* were removed, after some practice, down to the P_1's inclusive if the plastochron was at a late stage, or down to the P_2's inclusive if it was at an early stage. These operations were chosen because if the suggestion of Richards were correct, a removal of the P_1's should increase the angle I_1–I_2 to 90°, since normally the I_2's would have been caused to deviate from the plane at 90° to that of the I_1's by inhibitions from the P_1's. Similarly, a removal of young P_2's should increase the angle P_1–I_1 to 90°, provided that young I_1's were not yet determined: and the results will show that they are not determined. These operations did modify the phyllotaxis, though in unexpected ways, and accordingly further experiments were made in which the leaves were removed only down to a large pair of P_2's or a pair of P_3's inclusive. Also in four experiments they were removed down to a large pair of P_2's inclusive, and then one P_1 was also removed.

In *Dipsacus* the P_2's from the middle of the plastochron onwards, and all the older leaves of the bud, completely enwrap the younger pairs and stem apex, so that it is not possible, as in some plants, to keep any of the older developing leaves when operating: but some strong pairs of *mature* leaves were left to photosynthesize. When the youngest pair removed were older than young or medium P_2's, the stem apex was not exposed and it could not be seen how many more pairs remained. But the youngest pair removed was then recognized from its length, the P_2's having been found to range in length roughly from 0·25 to 0·9 mm. during the plastochron. The young leaves were removed under a binocular at magnification × 50 by cutting round their outer faces at the extreme base and then pressing them outwards. When the operations succeeded, the extreme bases of the cut-down leaves, even of the P_1's, could still be seen directly afterwards with their outlines unbroken all round. But they did not grow up, and after some days the bases of the P_1's and P_2's had disappeared. In young seedlings it is difficult to get at the youngest leaves, since the whole terminal bud is sunk deep down in a tube formed by the bases of the expanded leaves. But later, when in summer the seedlings are about 7 weeks old, the stem has grown and carried up the terminal bud just a little, and this was the stage at which the seedlings were operated upon. They do not flower in their first year. The buds were still only just above the level of the soil, and consequently the exposed stem apices and youngest leaves were very liable to be attacked by tiny mites and insects coming from the soil. But a thick ring of powdered derris placed all round the exposed apical parts was found to protect them fairly well, and to be quite harmless. Di-methyl-phthalate was found harmful even at a distance. The exposed parts were covered thickly with cotton-wool, and the plants were left usually for about five plastochrons, which took about 18 days in a greenhouse in summer. The buds were then pickled in spirit, embedded in collodion and sectioned as usual. Afterwards, under a projection microscope, either the sections were drawn or the median planes of successive leaf pairs were ruled on the image with a transparent ruler, and the angles between the successive planes were measured on the drawings or rulings.

4. THE MAIN EXPERIMENTS

Table 1 shows the angles between the planes of successive leaf pairs in the experiments, so far as they could be measured accurately, each angle being marked R for right (clockwise) or L for left. The spaces in the table for a few of the angles contain no figures, but are marked only R or L: this is because the leaves of the pairs adjoining these angles were too far from being opposite, so that the angles were not fully comparable with the others. Since the bases of the removed leaves disappeared or remained only as very small fragments, the angle from the youngest pair removed to the next younger pair could not be measured. Consequently, the next angle after this is the first angle recorded for each apex in the table. A difficulty in calculating the means of the angles is that sometimes in the experiments the genetic spirals reversed, as will be more fully described below. But another of the chief points which the table shows is that the mean angles increased, and a change in angle which goes beyond 90° and so constitutes a reversal is, in a way, a greater change and a greater increase than one which goes just to 90°. Consequently, it would be misleading to calculate the means only from the values of these reversed angles measured in the new direction, in

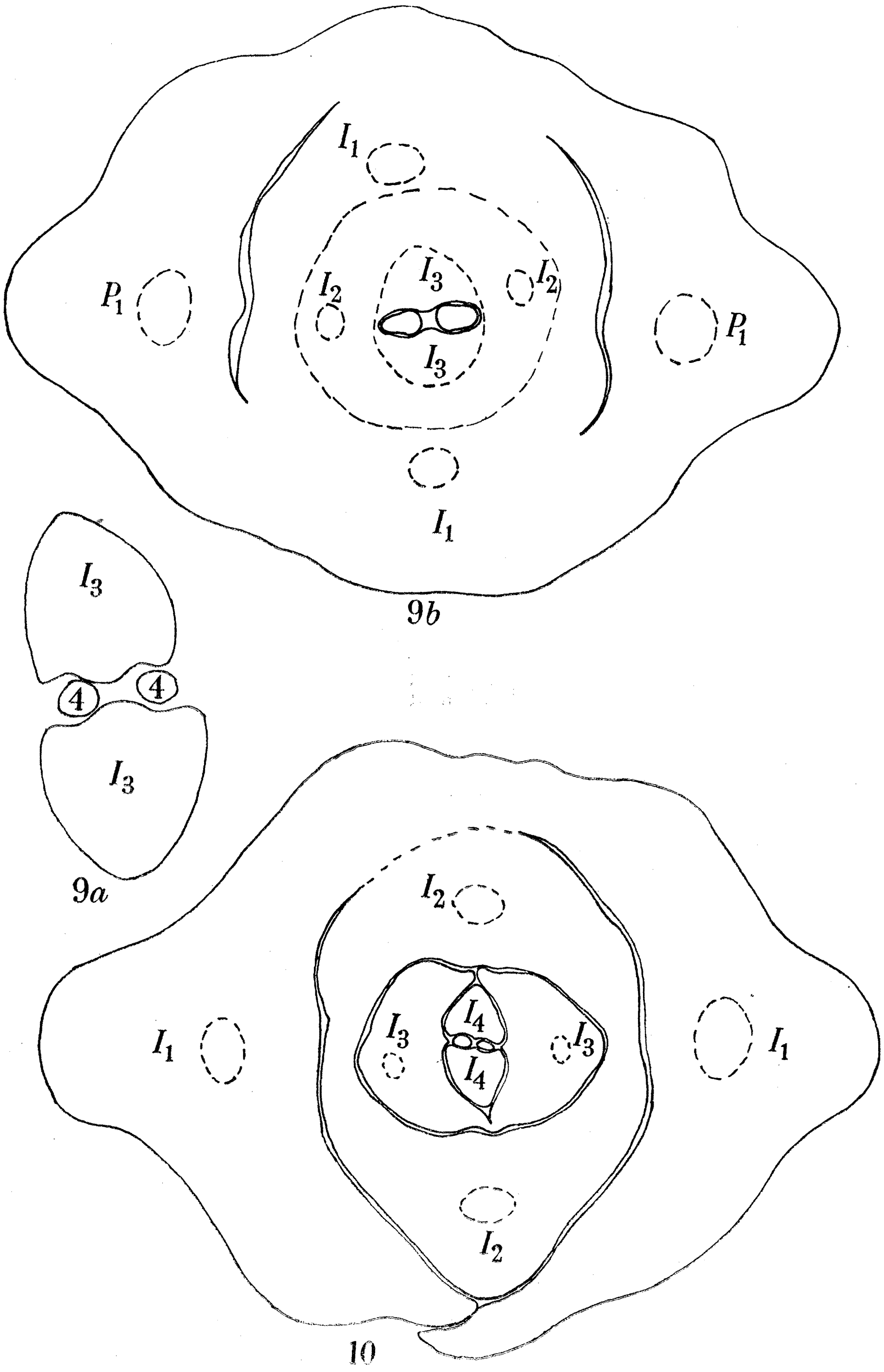


Figure 9. No. 41, P_2's removed (magn. *a*, × 53; *b*, × 23).

Figure 10. No. 68, P_1's removed (magn. × 23).

which they are less than 90°. So the means marked (*a*) have been calculated from the value of each angle measured in the same direction as that of the previous angle, which gives values over 90° for the reversed angles. These angles are recorded in the table, and also just below

Table 1. Results of experiments

Angles in degrees, left or right, between successive leaf pairs

Group 1, with leaves removed to P_3's inclusive: nine apices.

no. of apex	stage of plastochron at operation	original spiral	P_2–P_1	P_1–I_1	I_1–I_2	I_2–I_3	I_3–I_4
31	early	R	70 R	81 R	78 R	82 R	
32	late	L	81 L	71 L	80 L	78 L	82 L
33	early	L	69 L	87 L	68 L		
34	middle	L	77 L	64 L	87 L	82 L	
35	late	L	64 L	75 L	72 L	90	
36	middle	R	71 R	79 R	77 R	84 R	
37	late	R	75 R	84 R	70 R	97 R 83 L	
38	very late	R	73 R	83 R	86 R		
39	early	L	78 L	67 L	85 L	77 L	87 L
		means (*a*)	73·1	76·8	77·8	84·3	84·5
		means (*b*)	73·1	76·8	77·8	82·3	84·5

Group 2, with leaves removed to P_2's inclusive: twenty apices

no. of apex	stage of plastochron at operation	original spiral	P_1–I_1	I_1–I_2	I_2–I_3	I_3–I_4	I_4–I_5	I_5–I_6
14	early	R	83 R	75 R	76 R	81 R		
23	very early	R	88 R	87 R	78 R	93 R 87 L		
							105 L 75 R	
								90
30	middle	L	95 L 85 R					
				110 R 70 L				
					88 L	82 L		
40	late	R	81 R	78 R	89 R	80 R		
41	middle	R	81 R	86 R	93 R 87 L			
						85 L		
42	late	R	76 R	83 R	87 R	81 R	83 R	
46	early	R	81 R	85 R	88 R	86 R		
47	late	R	78 R	79 R	73 R	83 R		
48	middle	R	81 R	77 R	70 R	81 R	78 R	
49	early	R	80 R	78 R	90	88 R	80 R	
51	middle	R	74 R	76 R	77 R	79 R		
52	early	R	82 R	69 R	88 R	80 R		
53	early	R	80 R	77 R	81 R	72 R	75 R	78 R
54	very early	R	79 R	88 R	65 R	93 R 87 L		
55	middle	R	80 R	78 R	88 R	77 R		
57	very early	L	82 L	76 L	79 L	87 L		
59	very early	R	100 R 80 L					
				86 L	78 L	88 L	80 L	
60	very early	R	79 R	88 R	77 R	90	83 R	
62	middle	L	80 L	73 L	82 L	80 L	80 L	
63	very early	L	82 L	74 L	80 L	78 L	77 L	
		means (*a*)	82·1	81·1	81·3	83·2	82·3	84·0
		means (*b*)	80·6	79·1	81·0	82·6	79·0	84·0

Group 3, with leaves removed to P_1's inclusive: eleven apices

no. of apex	stage of plastochron at operation	original spiral	I_1–I_2	I_2–I_3	I_3–I_4	I_4–I_5	I_5–I_6
4	late	L	86 L	75 L	82 L		
10	very late	R	83 R	80 R			
16	late	L	85 L	72 L	75 L		
17	late	L	90	— L	— R	90	79 L
19	late	L	101 L 79 R				
				103 R 77 L			
					76 L	83 L	
20	late	L	79 L	78 L	70 L	85 L	
26	middle	R	93 R 87 L				
				90	78 L	88 L	
50	late	L	78 L	82 L	82 L	80 L	77 L
58	late	R	90	— L	— L	— R	102 R 78 L
61	very late	L	88 L	98 L 82 R			
					84 R	88 R	81 R
68	late	L	91 L 89 R				
				86 R	93 R 87 L		
						79 L	
		means (*a*)	87·6	84·9	80·0	84·7	84·7
		means (*b*)	84·9	80·2	79·2	84·7	78·7

Group 4, with leaves removed to P_2's inclusive and also one P_1 removed or destroyed: four apices

no. of apex	stage of plastochron at operation	original spiral	I_1–I_1	I_1–I_2	I_2–I_3	I_3–I_4	I_4–I_5
24	late	R	82 R	82 R	94 R 86 L	85 L	83 L
28	late	R	78 R	87 R	77 R	85 R	78 R
29	late	L	73 L	87 L	70 L	86 L	
66	middle	L	73 L	85 L	89 L	99 L 81 R	86 R
		means (*a*)	76·5	85·2	82·5	88·7	82·3
		means (*b*)	76·5	85·2	80·5	82·2	82·3

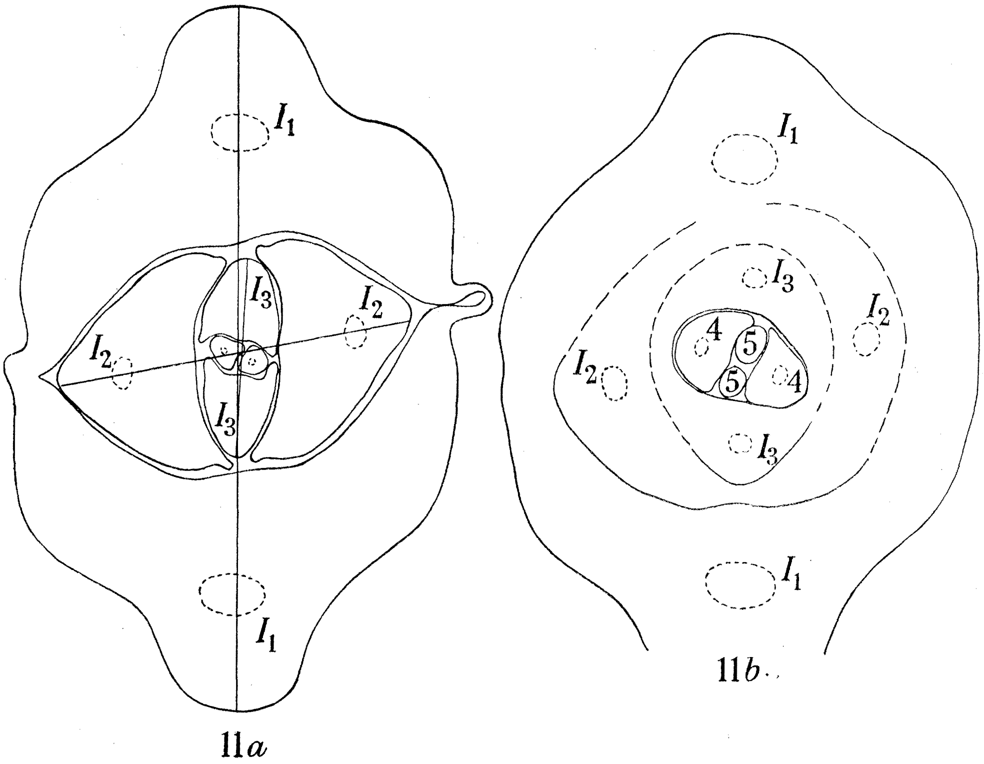
them the corresponding smaller angle measured in the new direction, which then becomes the direction for measuring the next angle, and so on. Thus the pairs of values in the table serve to mark the reversals. Also another set of means marked (*b*) has been calculated, for which the smaller of the paired values has been taken, though this seems less significant.

Drawings of sections of several of the apical buds are shown in figures 9 to 16, the sections of each apex being labelled alphabetically from above downwards. Because of the crater-like shape of the apical region, the outer leaves are often inserted higher than the inner. Broken lines are used to outline the median bundles and also to outline those young leaves or parts of leaves or of the stem apex which were seen rising up from a background of tissue and not sharply cut through below. The adjoining surfaces of leaves in contact are usually shown with two lines for clearness, although where the lines come close together there was regularly no chink between the leaves, which were very tightly packed in the bud.

It can be seen in the table that after all the operations the mean values (*a*) of the angles recorded were considerably greater than the normal mean value of 72°·7, with S.D. 0·66, except of course the angle P_2–P_1 recorded after removal of the P_3's, which was already laid down and could not change. The mean value of this angle, 73°·1, serves to confirm the mean of the normal angles already reported. It is remarkable that the increases of angle continued even for as much as five plastochrons after the operations. The increases were on the whole as might be expected, greater after removal of P_2's than P_3's, and greater still after removal of one or both P_1's. But exceptions to this are the mean angle I_2–I_3 after removal of P_3's, which was as high as 84°·3 in seven apices, and the mean angle P_1–I_1, after removal of P_2's and destruction of one P_1, which was only 76°·5, but in a group of four apices only. This angle P_1–I_1 was of course measured from the remaining P_1. The increases in the angles sometimes led to a phyllotaxis that was nearly decussate, for instance in figure 9, no. 41, a removal of P_2's, and in figure 10, no. 68, a removal of P_1's. These drawings should be compared with the normal in figure 1. Figure 19, no. 35, shows a single angle of 90° between the youngest two pairs of leaves, which followed rather surprisingly a removal of P_3's only.

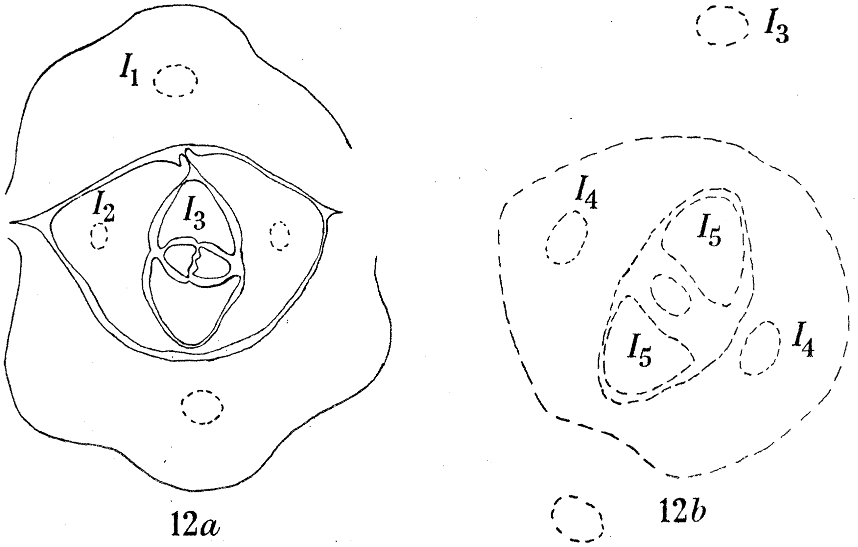
A striking reversal is illustrated in figure 11, no. 19, a removal of P_1's. The lines ruled through the I_1's, I_2's and I_3's make it clear that the reversal of the angle I_2–I_3 has brought back the I_3's almost exactly over the I_1's. The first angle, I_1–I_2, was itself reversed from the original direction of the spiral (left), which the second reversal restores. Measurements of angle can be found in the table. In figure 12, no. 26, also, another removal of P_1's, the angle I_1–I_2 is reversed from the original direction (right), but the spiral continues reversed. In figure 13, no. 23, a removal of P_2's, in which the spiral winds clockwise as far as the I_3's, there are two very late reversals, at the angles I_3–I_4 and I_4–I_5. The lines ruled through the I_2's and later pairs make this clear. There are also reversals in figure 10, no. 68, and in figure 16, no. 66, both at the angle I_3–I_4.

Altogether, amongst 177 new angles laid down after the operations, there were seventeen reversals, of which five were at the first angle recorded. In the groups with one or both P_1's removed, taken alone, there were nine reversals amongst fifty-seven angles.



11a

11b



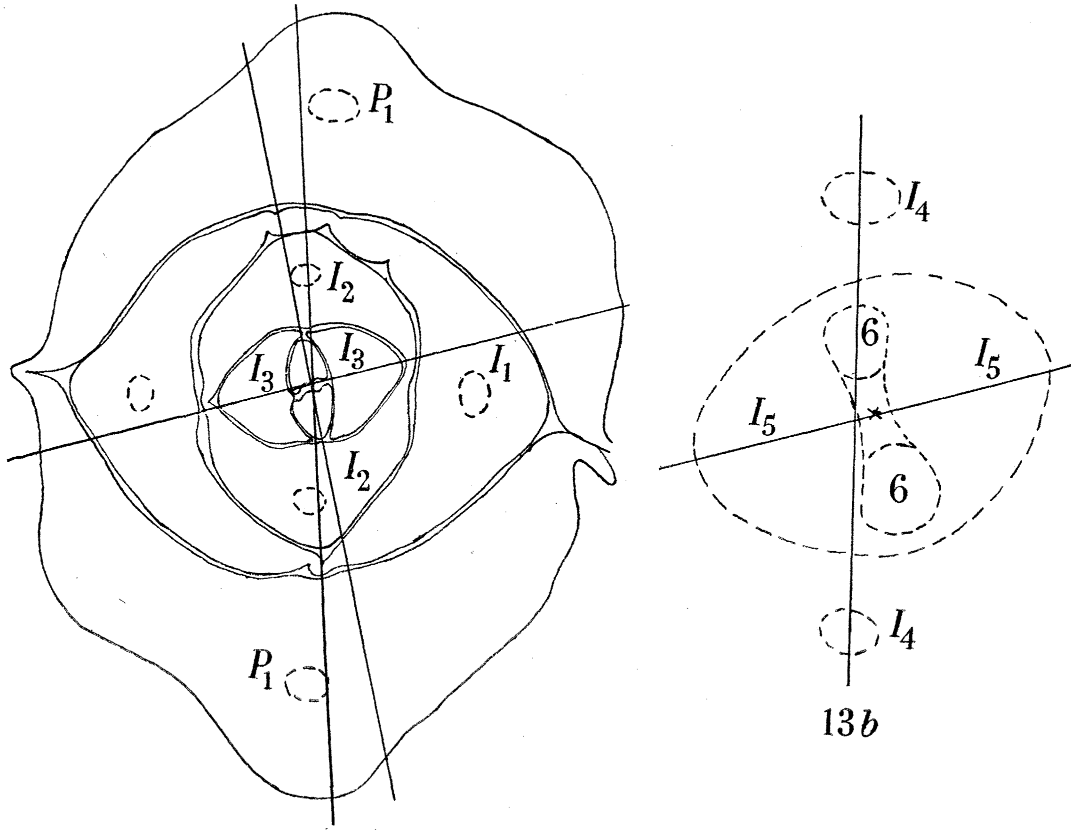
12a

12b

FIGURE 11. No. 19, P_1's removed (magn. *a*, *b*, ×23).

FIGURE 12. No. 26, P_1's removed (magn. *a*, ×16; *b*, ×53).

On the upward inhibition hypothesis of Richards it was to be expected that after removal of P_1's the angle I_1–I_2 would increase to 90°, since normally the I_2's would have been caused to deviate from a position at 90° to the I_1's by inhibition from the P_1's: but the angle I_2–I_3 and subsequent angles should not be changed. Similarly after removal of P_2's the angle P_1–I_1 should increase to 90°, provided that the I_1's were undetermined, but not the subsequent angles. After removal of P_3's there should be no changes at all on that hypothesis.



13*a*

13*b*

FIGURE 13. No. 23, P_2's removed (magn. *a*, × 19; *b*, × 60).

So the inhibition hypothesis does not account for the increases found in the angles later than I_1–I_2 after removal of P_1's, nor later than P_1–I_1 after removal of P_2's, nor for any of the increases after removal of P_3's.

The reversed angles are not only unexplained on the inhibition hypothesis, but contrary to it. For if the younger and older leaf pairs bordering these angles are called n and n–1, then pair n is situated more directly above pair n–2 than if it had been at right angles to pair n–1, although on the hypothesis pair n–2 inhibits pair n. This does not indeed apply to those five of the seventeen reversed angles which were the first angles recorded, since in relation to those five angles the pair which was n–2 had been removed; but it does apply to the other twelve reversed angles, and in figure 11, no. 19, the conflict with the inhibition hypothesis is especially striking.

It should, however, be noted that throughout the experiments, although the normal tendency of the phyllotaxis to deviate continually from 90° in the same direction was much weakened, as was shown by the increases of angle and by the reversals, yet something of this tendency persisted. For the mean values of the angles did not reach 90°, and the reversals were only occasional, even in the groups with one or both P_1's removed. As to the time of determination of the I_1's in this species, the increases in the angle P_1–I_1 after removal of P_2's, and the big convergence of the I_1's after destruction of one P_1 in no. 66, figure 16, show that certainly during the first half of the double plastochron at least, and probably for rather longer, the I_1's are not determined (see table 1 for the stage of plastochron at operation). Thus the time of determination is nearly the same as in *Lupinus albus*, where we found that I_1 is probably not determined until nearly the middle of the plastochron, which is single[*] (Snow, M. & R. 1933, pp. 398, 399; 1947, pp. 7, 15; 1948, p. 272.)

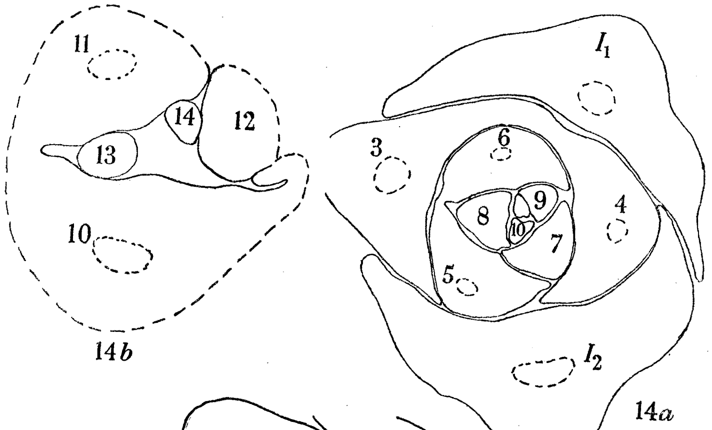


FIGURE 14. No. 22, P_1's removed (magn. *a*, × 11; *b*, × 40).

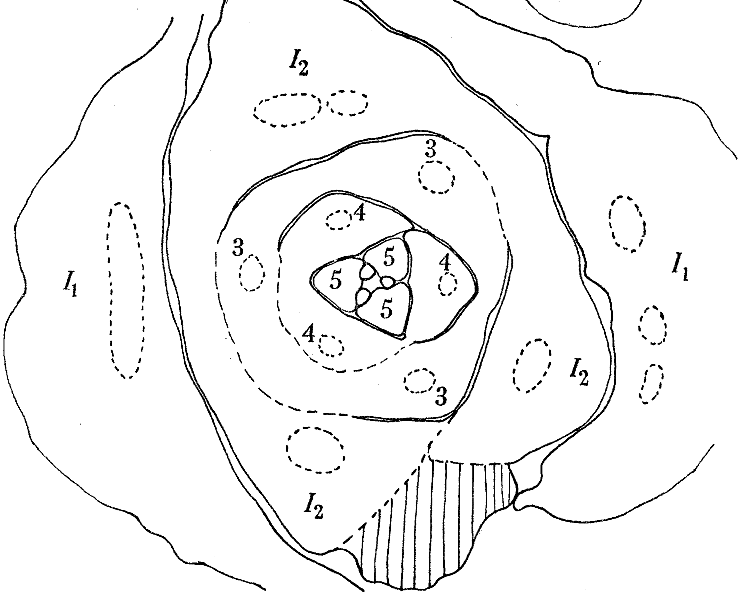


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FIGURE 15. No. 56, P_1's removed (magn. × 17).

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[*] Philipson (1949, p. 31) has criticized our interpretation of our experiments of undercutting presumptive areas of leaves in *Lupinus albus* (1947) on the supposed grounds that our conclusions concerning the time of determination of a leaf were based on operations that interfered with its presumptive area, so that the leaf concerned may have been determined partially, though not irreversibly. But actually those conclusions, like the present, were based on operations that did *not* interfere with (that is, enter) the presumptive area of the leaf concerned, as was clearly stated (1933, p. 398). Those operations were chiefly the eleven isolations of P_1 belonging to group 1 (1931, p. 17, p. 40 and figure 3). Also all except one of the undercut leaves (1947) were not I_1, as his statements imply, but I_2.

Besides the forty-four apices included in the table there were four others that were too different to be included. Two of these, in which the P_1's were removed or one P_1 was destroyed, had leaves irregularly paired; one, figure 14, no. 22, in which the P_1's were removed when of medium size, became spiral, and one, figure 15, no. 56, in which the P_1's were removed when large, formed whorls of three after the I_1's. The factor leading to the spiral shown in figure 14 cannot be discovered, but may have been some slight accidental injury. The operation itself would not be expected to lead to a spiral, though a diagonal split of the stem apex would be likely to do so, as in decussates.

But the development of the three-whorled apex shown in figure 15 can probably be explained as follows. When this apex was examined 7 days after the operation, the I_1's appeared to be wilted and irregular in shape, as they still are in the drawing, presumably because the covering of cotton-wool had not been close enough. In the drawing it can be seen also that the two I_2's which are closest together are joined by some unorganized tissue which is shown cross-hatched. It therefore seems probable that an original I_2 collapsed in the period of wilting, and two new I_2's were then formed from its base, making a whorl of 3. The divided bundle of the other I_2 suggests that it nearly did the same. It may be noted that after the I_2's the leaves of alternate whorls are almost exactly superposed.

Since in the main experiments the increases of the angles between leaf-pairs continued for several plastochrons after the operations and were not in any very obvious way junctional consequences of the operations, it was thought that they might be due to some persisting physiological change in the conditions of growth, and accordingly some attempts were made to change these conditions in other ways. In two apices, nos. 62 and 63 of the table, the P_2's were removed in the usual way, but then much more light than usual was admitted, with due precautions, to the stem apex and youngest leaves. But the subsequent changes of angles in these two were not in any way exceptional.

More effective was the inverse stimulation by gravity of two plants. These plants were not operated upon, but the pots containing them were propped in the inverse position with a sheet of white paper below them as reflector. The older leaves were also partly lit from above, since they projected beyond the pot. The apical buds were much retarded in growth by the inversion, and after 16 days they had nearly stopped growing. Then the pots were turned upright, the apical buds began slowly to grow again, and after a further period of about 25 days they were pickled. At the start of the inversion an outer pair of leaves of the bud had been marked and measured, and by calculating from the length of this marked pair, it was estimated which had been the youngest pair at that time. From that pair onwards the angles between successive pairs were measured on drawings or rulings of bud sections down to the youngest pair finally visible in no. 1 and the youngest but one in no. 2, and the following values in degrees were recorded:

In no. 1:	72	67	69	69	72	69	—
In no. 2:	72	66	68	64	63	64	70

There can be little doubt that the inversion had *decreased* the angles below the normal mean of 72°·7, especially as the first angles recorded were probably laid down before the inversion had produced its full effect. These two apices will be further discussed below.

5. Further observations and suggested explanations

The evidence of the experiments reported is decidedly against the explanation of bijugy which they were designed to test, and it therefore needs to be considered whether the bijugy of *Dipsacus* can be explained in some other way which is consistent with the experiments. It must first be pointed out that there is evidence of an active whorl-forming process in *Dipsacus*, similar to that which the writer found in decussate apices of Labiatae (1942), and which he showed to comprise one factor for equalizing the levels of the positions in which leaves are formed, and another factor for equalizing the lateral spacing of these positions. For the equalizing of levels in *Dipsacus* is shown strikingly in figure 16, no. 66, in which the I_1's converged strongly (29°) towards the position of a destroyed P_1, but the I_2's arose very accurately at the same level, though junctional factors alone would have caused the I_2 in the larger angle to arise earlier and lower. The equalizing of lateral spacing is shown in the three-whorled apex, figure 15, no. 56, in which the leaves of the first whorl of three are spaced very unequally, but those of the later whorls fairly equally. Several other examples of the whorl-forming tendency were found in *Dipsacus*, although the experiments were not designed to test for this tendency, as were those on the Labiatae. Incidentally it may be noted that the whorl-forming tendency in the plants of these families

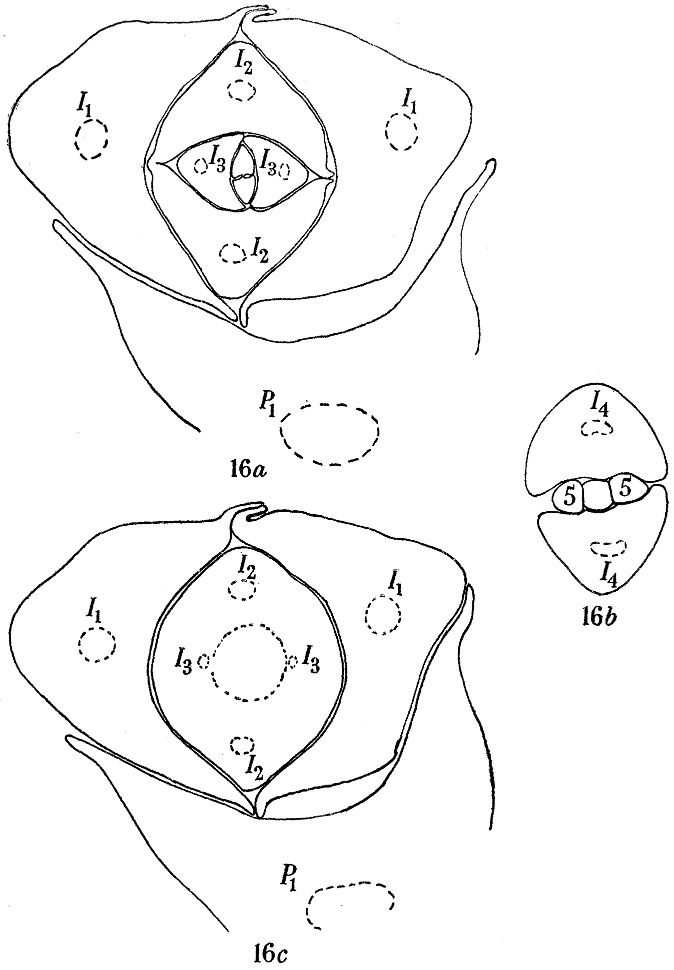
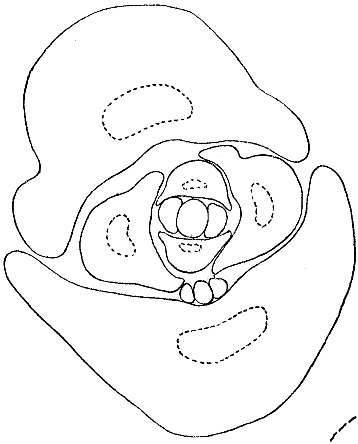


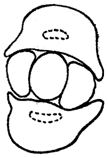
Figure 16. No. 66, P_2's removed and one P_1 destroyed (magn. *a*, *c*, × 12; *b*, × 40).

is *not* a tendency to form leaves in pairs, since it acts similarly on groups of three or more leaves, if the junctional factors alone would have caused those leaves to arise nearly in a whorl. The fact that normally in these plants the vegetative whorls contain two leaves can be explained on junctional grounds. These matters and the experimental production of spirals in decussate species were discussed more fully before (Snow 1942).

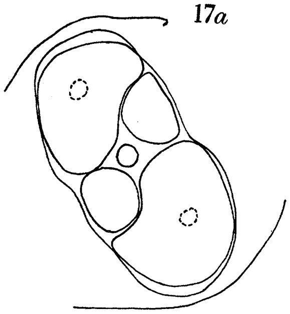
The formation of whorls, therefore, in *Dipsacus* and in the decussates is an active and primary process of some kind and is not merely a geometric consequence of the even numbers of the parastichies, as Church maintained (1904). Accordingly the problem of the bijugy of *Dipsacus* is how it is that the angles between successive leaf pairs deviate constantly from 90°. One factor that may play a part in bringing this about is suggested by comparison with seedlings of an exceptional labiate, *Salvia horminoides*, which form close and clearly bijugate rosettes. Two drawings of a favourable section through the apex of a rosette are shown in figure 17*a*, *b*, at different magnifications. The plastochron is at a late stage, the stem apex is elliptic, and the next pair of leaves is being or has been determined on the narrow ends of the ellipse. So it is clear that each leaf of this next pair will make contacts 1, 1 and 2—that is to say that it will make contact at its base not only with the leaves of the next older pair (contacts 1 and 1), as do the youngest leaves of straight decussate apices, but also with a leaf of the second older pair (contact 2). The making of contact 2 is naturally due to the smaller arc covered by each leaf.



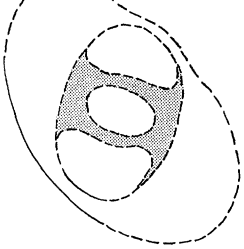
17*a*



17*b*



18*a*



18*b*

FIGURE 17. *Salvia horminoides*, normal apex (magn. *a*, × 38; *b*, × 54).

FIGURE 18. *Dipsacus laciniatus*, apex of an inverted plant (magn. *a*, *b*, × 40).

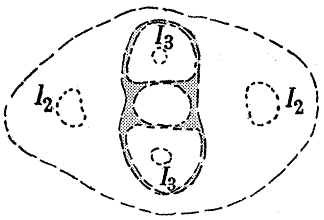
It can therefore readily be understood on the basis of a junctional and space-filling theory of leaf determination that the phyllotaxis of these rosettes deviates from straight decussation.

Indeed the change is quite similar to the transition common in other seedlings from distichy to a spiral with contacts 1 and 2, which is due to a fall in leaf arc and is well described by van Iterson (1907). The explanation of the change is clear if one considers the boundary line formed by the upper margins of the bases of the young leaves round the stem apex. Thus in figure 17 of *S. horminoides*, since the parts of the stem apex from which the new pair of leaves will arise make contact with the leaves of pair 2, the position which is the lowest for each new leaf, and which therefore will first become available for it, is not directly over the centre of leaf 2, where the circular base of that leaf comes nearest to the growing-point, but a little to one side, in one of the corners between leaf 2 and a leaf of pair 1. Accordingly the new leaf deviates a little to one side, and the equal spacing factor of the whorl-forming process ensures that the other leaf of the new pair deviates in the same sense. It is also clear enough that the deviations of successive pairs will continue in the same sense.

In *Dipsacus*, on the other hand, it seems at first that the deviations of the leaf-pairs from straight decussation cannot be explained in a similar way, since each new leaf makes contact only with the next older pair. But then the shapes of the leaf bases are very peculiar and far from being simply circular, since the bases of each pair unite by a low rim before the end of their first plastochron, as was described in § 2, figures 2 and 3. Moreover in observations of the *Dipsacus* apices from which pairs of large P_1's were removed, it often appeared that the boundary formed round the stem-apex by the bases of these P_1's with their united rims was far from being circular or elliptic, but distinctly showed four sides and four corners. So it seems quite possible that the united rims act as another pair of contacts, and that the positions which normally first become available for the next pair of leaves, if their arcs are small enough, are nearly the corner positions, as in *Salvia horminoides*, and not the median positions at right angles to the previous pair.

On this basis an explanation can be suggested for the increases of the angles after the operations, though something more is needed to explain the occasional reversals. For it is quite possible that when the leaves of the buds were all, or nearly all, removed, the arcs of the leaves formed subsequently were slightly larger than the normal, and that in the inverted plants they were slightly smaller than the normal. The increase of arc would then increase the angles between the leaf-pairs by preventing the new leaves from fitting so closely into the corner positions, whereas the decreases of arc would decrease these angles by enabling the new leaves to fit more closely into those positions. There are indeed some indications that the leaf arcs were larger after the operations and smaller after the inversions. Thus figure 19 is of a favourable section through the stem apex of no. 35, one of the plants operated upon. It shows a stage slightly after mid-plastochron, and one can see that the arcs of the youngest pair of leaves, which are the I_3's, are already so large that they could not easily be fitted into corner positions between the next older pair and their united rims. This section was lucky, since the angle had just risen to 90° (see the table).

Figure 20, no. 41, is at a rather later stage of the plastochron, and only the inner contour line of the second pair, which were the I_3's, is shown, since the section was below their outer insertions. It can be seen again that the youngest pair is too large to occupy corner positions. The angle I_3–I_4 was 85° left. Figure 21, no. 47, shows an apical section made at the very end of a plastochron, when the youngest pair had already united. The stippled area within the contour lines of the youngest pair is all apical tissue, and appears markedly four-sided. The central ellipse is a small apical dome rising up from this background. Figure 22, no. 42, shows an earlier stage at about mid-plastochron. Here it looks as if the bases of the youngest pair, which are the I_5's, could have deviated further into the corner positions, but they may not yet have grown to occupy the whole of the area over which they were determined, which is the critical area on a space-filling theory. The angle I_4–I_5 in this apex was measured from other sections as 83° R.



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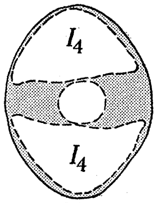

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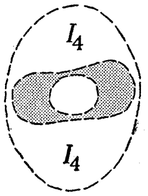

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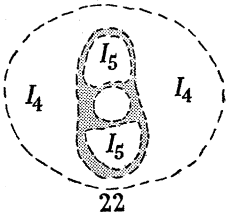

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FIGURE 19. No. 35, P_3's removed (magn. × 54).

FIGURE 20. No. 41, P_2's removed (magn. × 40).

FIGURE 21. No. 47, P_2's removed (magn. × 40).

FIGURE 22. No. 42, P_2's removed (magn. × 40).

A great contrast with these apices and with others of the plants operated upon was provided by the apices of the two inverted plants with small angles. Two consecutive sections of the first of these are shown in figures 18*a*, *b*. The higher section, figure 18*a*, has just cut the tip of the apical dome, and has cut the youngest pair just above their insertions. Judging from this section alone, one would have thought that the contacts of the bases of the youngest pair were 1, 1 and 2, as in *S. horminoides*; but the next lower section shows that the second pair are united by rims, and that each leaf of the youngest pair makes contact at its base with the next older pair, and with their united rims only, and that it fits very closely into one of the corner positions of the boundary line. So in this apex it does seem that the united rims of the 2's have acted as contact members in the same way as the 3's in *S. horminoides* (contact 2), and this forms a strong piece of evidence in favour of the explanation of the normal bijugy as due to a contact of the 1's with the united rims of the 2's. But many more observations are needed to discover how generally this factor may be at work.

Another factor which may play a part in causing the normal bijugy of *Dipsacus* is pressure exerted by the upper parts of the second pair of leaves (the P_2's of the operations) on the sides of the stem apex, which they touch in the way described in § 2. For there is evidence from the experiments of Němec (1903) that pressure on part of a stem apex tends to inhibit the formation of a leaf by that part, as might indeed be expected. This factor may act together with the factor due to the spatial relations of the leaf bases already discussed in causing the bijugy, since the two factors are quite compatible. It should not be confused with Schwendener's hypothesis of a shifting of *existing* young leaves by lateral pressures between them. The contact of the upper parts of the P_2's with the stem apex was found to persist through the first half of the plastochron, or a little longer, and this is the right time for influencing the positions of the pair about to arise (the I_1's of the operations), since the evidence showed that the I_1's were not determined until soon after mid-plastochron. It can be understood that the pressure of the P_2's, by tending to inhibit the formation of leaves on the parts of the apex directly above them, would cause the I_1's to deviate a little from the plane at right angles to the P_1's, and so to deviate from straight decussation.

This is exactly the way in which physiological upward inhibitions are supposed by Richards to act, and it might therefore be thought that the explanation based on pressure from the P_2's must fail to explain the long continuing increases of angle after the operations in just the same way as does the hypothesis of Richards. But this is not so, since the pressure exerted by the P_2's on the apex may depend on the pressure which they themselves receive from the older leaves outside them. Consequently, when in the operations these outer leaves, and usually the P_2's themselves, were removed, the pressures exerted afterwards on the apex by the successive pairs, as each became in its turn the second oldest pair, may have been less; and this would explain the long continuing increases of angle, and the increases after removal of the P_3's only.

This suggestion is supported by the following observation. When the outer leaves were removed, the P_2's were regularly found pressed tightly together along their inner surfaces, and then sometimes the buds were covered up and left for a few hours because bleeding from various cut surfaces made it difficult to see. When the buds were uncovered again, the P_2's were often found to be no longer pressed so tightly together, so that presumably they were not pressing so hard on the stem apex either.

But the reversals of angle in the experiments can be explained neither on the basis of the spatial relations of the leaf-bases alone, as was pointed out above, nor on the basis of the pressure from the P_2's alone. For if the latter were the only factor causing the normal deviations from decussation, then it would be expected that when this pressure was diminished or abolished, the angles would increase up to 90°, but not reverse. The reversals can, however, be explained in terms of both these factors working together, and it seems worth while to set out this explanation briefly, in order that it may not seem that both these factors are disproved because the reversals cannot be explained on the basis of either factor alone.

The explanation can readily be understood if one starts from a comparison with *Salvia horminoides*, with its contacts 1, 1 and 2 (figure 17). In that species, as was pointed out, the angles deviate from 90° because in each new pair of leaves, which will be called *n*, each leaf arises in a corner position of the boundary line, between a leaf of pair *n*–1 and another of pair *n*–2. Further, when once the deviations have started, pair *n*–1 is not at right angles to pair *n*–2; and consequently, of the two corner positions in which each leaf *n* might arise, one is wider than the other. So leaf *n* arises in the wider gap, and thereby the sense of the deviation from 90° is kept constant.

In *Dipsacus* it was suggested that the contacts of pair *n* with the united rims of pair *n*–1 act similarly to the contacts with pair *n*–2 in *Salvia horminoides*, and so cause the angles to deviate from 90°. But the boundary line formed by the united rims in *Dipsacus* does not rise up in the middle towards the summit of the apex nearly so much, if at all, as does the boundary line formed by pair *n*–2 in *Salvia horminoides*. Consequently, the two corner positions in which leaf *n* might arise are not so sharply differentiated into a wider and a narrower space, though they may differ slightly; and so far as this factor alone is concerned the sense of the deviation is not likely to be kept so firmly constant. But if normally in *Dipsacus* the pressure from the upper parts of pair *n*–2 (the P_2's) on the stem apex also plays a part in making the angles deviate from 90°, this factor will help to keep the sense of the deviation constant. For when the deviations have started, the greatest pressure from each successive pair *n*–2 will come on the apex a little towards one constant side of the plane at right angles to the plane of pair *n*–1. Consequently, when in the operations the pressure from each pair *n*–2 is abolished or diminished, this factor will no longer be at work, and the new pairs of leaves may arise in those corner positions in which they do not normally arise, thereby reversing the angles.

However these suggested explanations are far from being firmly established, and many more observations are needed to establish how generally either or both of the factors suggested may be at work in causing bijugy. In particular it needs to be observed whether in the other Dipsacaceae there are similar united leaf rims, and whether the P_2's touch the stem apex similarly. In *Scabiosa caucasica* the writer has found the rims of the youngest pair almost united near the end of a plastochron.

It was pointed out that bijugy is caused by another factor in *Salvia horminoides*, and it remains possible that in species of some other families bijugy may be caused by upward physiological inhibitions, as suggested by Richards, though in *Dipsacus* the evidence is against this. In any case, the factor already mentioned which equalizes the spacing of the members of a whorl may be a physiological inhibition or repulsion of some kind, but one that acts only between leaves that are about to arise at the same level, or very nearly so.

The idea that several factors may cause bijugy, and that sometimes two such factors may act together in the same plant, need not seem surprising, since bijugy in rosette plants has a clear functional value. So it is quite probable that natural selection may have worked up several different ways of bringing it about.

I am indebted to my wife for help in finding out how to operate on *Dipsacus*, and for a valuable discussion of the interpretation.

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