The Structure of the Starch Layer in the Glossy Petal of Ranunculus.

II. The British Species Examined.

BY

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With nine Figures in the Text.

INTRODUCTION.

IN a former paper (4) it was shown that in the petal of *Ranunculus Ficaria* the starch layer, apparently two to three cells deep as viewed in transverse section (Fig. 1, *st.*), is in reality composed of only a single row of cells, the false appearance being due to the peculiar oblique growth of these cells as viewed in longitudinal section (Fig. 2, *st.*). Incidentally the petals of the three common buttercups of our meadows and pastures, viz., *R. acris*, *R. bulbosus*, and *R. repens*, were also examined for comparison. The starch cells have likewise in these species the oblique character of growth, but with this striking difference, that the direction of the slope is the reverse of that shown in the petal of *R. Ficaria*. This suggested that considerable variation may occur in the structure of the starch layer in the genus, and the British species have now been examined with this end in view.

All the British species of Ranunculus have yellow and glossy petals with the exception of those belonging to the Batrachium section—the white water-buttercups—which consequently are not dealt with in this paper. The glossy species as described in Bentham and Hookers' *Handbook of the British Flora* (seventh edition 1930) number thirteen. *Ranunculus reptans* L. has since been raised to specific rank (1, p. 21). All have been examined except this last, the petal of which is not likely to differ materially in structure from that of its close ally, the common and variable *R. Flammula*.

For the supplying of flowers of the rarer British species, such as *R. ophioglossifolius* and *R. flabellatus*, I am indebted to Mrs. Foggitt and Lady Davy. Again, through the kindness of Professor J. H. Priestley, the microtome work in connexion with this paper has been carried out by his

laboratory steward, Mr. A. Millard. To all the above I wish here to tender my grateful thanks, and especially to the last mentioned for the care and interest he has taken in the cutting of the sections.

Technique.

Mature petals of Ranunculus are usually such flimsy structures as to render the cutting of their sections without distortion, especially in the longitudinal direction, a matter of considerable difficulty. Petals from flowers in the full-bud stage, that is just before expansion, can be dealt with more easily. Such material has consequently been largely used in this investigation. How far, it may be asked, will the structure of the slightly immature petal differ from that of the fully expanded one? From a detailed examination of the petal of *R. Ficaria* at all stages of development we think the difference to be but slight. When slope is present in the starch cells, its steepness may be a little augmented on full expansion.

The material was fixed in Flemming's chromo-acetic-acid solution, and the sections cut moderately thick, 10 to 12 μ. If cut thinner the starch granules are apt not to be retained *in situ*. The stains used were either Hanstein's aniline violet or light green and safranin. The former is quite good and simple to use, but has the disadvantage of soon fading. The latter, a double stain, though more difficult to manipulate, is fairly permanent, colouring the cell-walls green and the starch granules pinkish.

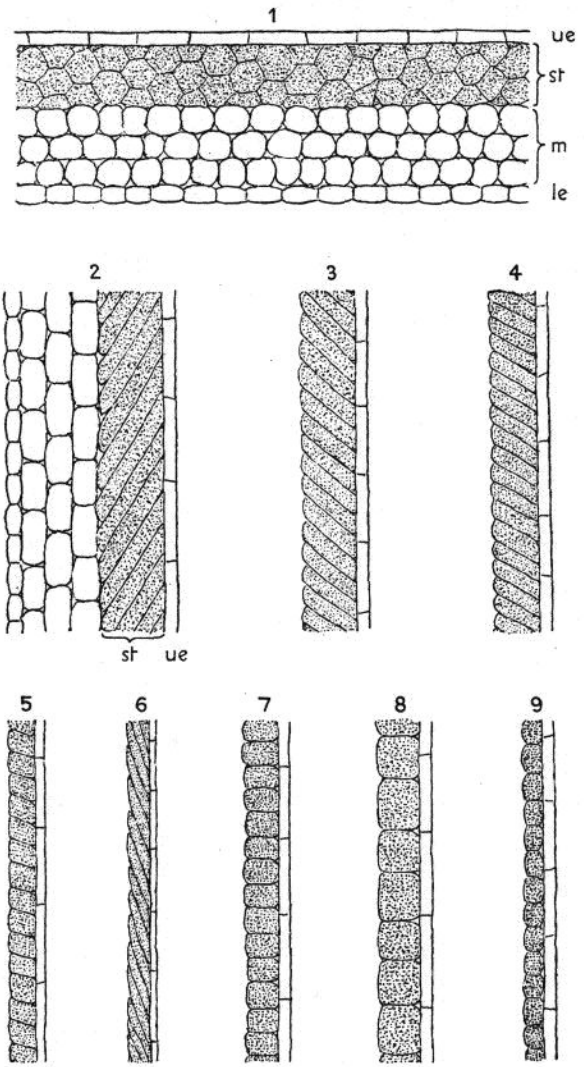
Descriptive.

The species are taken in alphabetical order and the slope and direction of the starch-cells briefly described. In each instance the observations made refer to the middle part of the glossy (starch-containing) area of the petal. When slope is present it tends to be less pronounced at both the distal and proximal borders of this area. The degree of the slope is indicated roughly by the size of the acute angle the starch cell makes with the upper (ventral) surface of the petal. For example, an angle of 30 degrees indicates twice as much slope as one of 60 degrees. Regarding the direction of the slope the terms 'upwards' and 'downwards' refer respectively to the apex and base of the petal, and the terms 'outwards' and 'inwards' to the upper and lower surfaces. The measurements of the depth of the starch layer are approximate only and given for the sake of comparison.

R. acris L. The starch cells have a decided slope of about 45 degrees directed upwards and inwards. Depth of starch layer, 38 μ.

R. arvensis L. The slope is only slight, the angle being about 60 degrees. The direction is also upwards and inwards. The depth of the starch layer, 22 μ.

R. auricomus L. No slope is shown. The starch cells are almost



FIGS. 1–9. 1. Transverse and Fig. 2, longitudinal section of the petal of *Ranunculus Ficaria* L. 3–9. Longitudinal sections of various petals showing only upper epidermis and starch layer. 3. *R. bulbosus* L. 4. *R. repens* L. 5. *R. sardous* Cr. 6. *R. parviflorus* L. 7. *R. auricomus* L. 8. *R. Flammula* L. 9. *R. sceleratus* L. *u.e.*, upper epidermis; *l.e.*, lower epidermis; *st.*, starch layer; *m.*, mesophyll. Cell contents are only indicated for the starch layer. Magnification × 150. Figures are all semi-diagrammatic.

cubical in shape, with a slight tendency to palisade arrangement. Depth of starch layer, 30 μ (Fig. 7).

R. bulbosus L. The slope is pronounced, directed upwards and

inwards, the angle being about 30 degrees. Depth of starch layer, 45 μ (Fig. 3).

R. Ficaria L. The slope is pronounced, with an angle of about 30 degrees, and is directed downwards and inwards. Depth of starch layer, 60 μ (Fig. 2).

R. flabellatus Desf. (*R. chaerophyllos* L.). This species can only claim to be British through occurring wild in the island of Jersey. It has a starch layer similar to that of *R. bulbosus*, but the slope may be a little less pronounced.

R. Flammula L. The starch cells have no slope, and are slightly elongated in the longitudinal direction parallel to the surface of the petal. There is no indication of palisade arrangement. Depth of starch layer, 38 μ (Fig. 8).

R. Lingua L. No slope. Starch cells similar to those of the previous petal. Depth of starch layer about the same.

R. ophioglossifolius Vill. This species is extremely rare in the British Isles, and we have only been able to procure a few specimens from one source. The results obtained suggest that the starch cells have no slope, but this requires confirmation when more material is available. Depth of starch layer, 22 μ.

R. parviflorus L. This minute petal gave evidence of having starch cells with a pronounced slope directed upwards and inwards. The oblique character of these cells would appear to be even greater than in *R. Ficaria*, though directed in the opposite way. Depth of starch layer, 15 μ (Fig. 6).

R. repens L. The slope of the starch cells is fairly similar to that of *R. acris*, but perhaps a little more pronounced. The direction is the same. Depth of starch layer, 38 μ (Fig. 4).

R. sardous Crantz. (*R. hirsutus* Curtis.) The slope is not at all pronounced, though somewhat more apparent than in *R. arvensis*, and similarly directed. Depth of starch layer, 22 μ (Fig. 5).

R. sceleratus L. Slope is absent. The starch cells as viewed in longitudinal section are slightly elongated parallel to the surface of the petal and inclined to be rounded in contour. Depth of starch layer, 18 μ (Fig. 9).

Comments.

From these observations it is obvious that the oblique growth of the starch cells is not an invariable accompaniment of gloss. Further it would seem that the intensity of the gloss is not exactly proportional to the slope of the starch cells. It is true that the petal of *R. Ficaria* with marked slope is very glossy, but not perhaps more so than that of *R. Flammula* with straight starch cells. It is just possible that slope may augment colour-depth though increasing the opacity of the petal. In the British

species, taken as a whole, the petals with little or no slope are less intensely yellow than those with pronounced slope, but this may be due actually to a less amount of pigment. From the figures given it is apparent that in petals more or less of the same size the depth of the starch layer increases with the slope of the cells; and this in itself would bring about greater colour-intensity in the petals with slope, the amount of pigment (which is restricted to the epidermal cells) remaining the same. *R. bulbosus*, for example, with more slope than *R. acris* or *R. repens*, has a deeper starch layer. *R. Lingua*, with the largest petal of all the British species of this genus, has a starch layer no deeper than that of *R. acris*. Probably the more the glossy area assumes the appearance of enamel, the deeper will be found the starch layer and the more inclined the starch cell.

With greater certainty one might draw the inference from these observations that the oblique growth of the starch cells is an advanced feature, and that primitively these cells were not so differentiated. The species *R. sceleratus*, *R. auricomus*, *R. Lingua*, and *R. Flammula*, with no slope, would appear to be less highly evolved than *R. acris*, *R. bulbosus*, and *R. repens* with decided slope. The petals of the former group have naked nectaries, or almost so, while the latter have these glands covered by marked scales. A naked nectary pit is presumably primitive to a covered one. Kumazawa in his morphological study of Japanese species of Ranunculus (2) considers *R. sceleratus* as probably one of the most primitive representatives of the genus.

The structure of the starch layer in *R. sardous*, *R. arvensis*, and *R. parviflorus* is not altogether as might have been expected. On account of these species having scales to their nectaries and tuberculate or prickly carpels, and being annuals, they might be regarded as more advanced than, for example, *R. acris*, and I was prepared to find their petals with pronounced sloping starch cells. *R. parviflorus* certainly appears to have such, but in the other two species, especially *R. arvensis*, the slope is only slight. These three species are generally put in the section Echinella, characterized by having tuberculate carpels. Quite possibly this style of achene may have evolved more than once independently in the genus. *R. ophioglossifolius* is interesting in this connexion. Its achenes are minutely tuberculate, and for this reason it has also been placed in the Echinella section; but present-day systematists are more inclined to regard it as belonging to the Flammula group of species. The structure of the starch layer as far as we have been able to examine it points that way.

The most striking outcome of this research on the structure of the starch layer is undoubtedly the opposite direction which the slope takes in the petal of *R. Ficaria* to that assumed in the other species. This is in keeping with the usual opinions held regarding the classification of the genus. *R. Ficaria* certainly in its general morphology stands apart, and

the early systematists recognized this so much as to place it in a separate genus, *Ficaria*. Perhaps a comparison might be drawn here between the occurrence of both right- and left-handed twining plants, and right- and left-spiralled snail shells. Of these opposites one form is usually much more common than the other. Dextrose twiners are more numerous than sinistrose, and dextral shells more so than sinistral. Probably a complete investigation of the genus Ranunculus would show that petals with the starch cells directed upwards and inwards are much more numerous than those with these cells sloping in the opposite way—at present only known in *R. Ficaria*.

The reason why these starch cells in development should assume this oblique form is obscure and so becomes a matter of speculation. Given a tendency for these cells to grow in length at right-angles to the surface of the petal, it is possible to imagine their being pulled out of the perpendicular into a slanting direction through the extension of the ordinary mesophyll cells lengthwise parallel to the surface. The study of the development of the petal of *R. Ficaria* suggests some such causal explanation (4, Figs. 4–7). But why the pull should be in one direction in *R. Ficaria* and in the opposite direction in the other species is not thereby explained.

Cells of this slanting type are distinctly unusual in plant tissues. They are known to occur occasionally in assimilating tissue. I am indebted to Professor Priestley for calling my attention to the literature. Pick (5) was the first to observe these cells, and thought that they assumed such a position in the mature leaf when illuminated by oblique light rays. Later investigators, including Haberlandt, do not subscribe to this view, and Liese (3) who has more recently studied the influence of light on the orientation of assimilating cells finds that the incidence of the rays may influence during development the direction taken by the palisade cells, but ceases to have any effect at maturity. These oblique palisade cells are, however, more of interest here as another example of an unusual form of tissue than as suggesting that the obliquity of the petal starch cells has any direct connexion with the incidence of the light rays. The petals during their development are not exposed to light, being well covered by the sepals.

From this study, as far as it has progressed, one receives the impression that the tendency in the genus has been to increase the opacity, and so the colour-intensity, of the petal by deepening the starch layer. This has been brought about by the cells assuming a palisade arrangement; but instead of their being developed perpendicular to the surface of the petal they have been pulled as it were during growth into an oblique direction.

Summary.

1. The slanting of the starch cells of the petal is not universal in the British species of Ranunculus. The cells have—

(*a*) no slope in *R. auricomus*, *R. Flammula*, *R. Lingua*, *R. sceleratus*, and probably *R. ophioglossifolius*.

(*b*) A slight slope in *R. arvensis* and *R. sardous*.

(*c*) A decided or pronounced slope in *R. acris*, *R. bulbosus*, *R. repens*, *R. flabellatus*, *R. parviflorus*, and *R. Ficaria*.

2. The direction of the slope in *R. Ficaria*, opposite to that in the other species with oblique starch cells, is emphasized and discussed.

3. Slope is regarded as a derived and not a primitive feature of these petals.

4. A causal explanation of the slope is tentatively suggested. It may be initiated when the starch cells tend in the course of evolution to assume a palisade character, i.e. to grow at right-angles to the surface of the petal. A pull on them during development by the extension of the adjacent cells lengthwise may cause the obliquity.

5. The tendency in the genus has probably been to increase the opacity, and so the colour intensity of the petal by deepening the starch layer.

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