STUDIES IN THE DEVELOPMENT of the SEXUAL TISSUES IN THE GENUS RHODODENDRON

by

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I. INTRODUCTION.

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The genus Rhododendron has been considered from time to time by various investigators from several different angles, but mainly from the anatomical, morphological, and systematic view-points.

While SAX (35) and others have reported on the cytology of a number of species of Rhododendron it was felt that a survey of the genus, especially with regard to species not previously investigated, would be of value. During the course of the preliminary work in this connection considerable difficulty was experienced in determining the exact time when the sporogenous tissue in the male and female organs was initiated. It was found on the examination of a large number of plants comprising many different species of Rhododendron that there was a wide separation in time between the initiation of the male and female sporogenous tissues - a period in the majority of cases extending over several months. Furthermore, irregularities of considerable extent appeared in a number of species.

It became abundantly clear that attention to these phases of the cytological problem would yield results of greater fundamental interest than routine counts of the chromosome number; hence the main thesis /

thesis to be developed here turns on the initiation of the sexual phase of the plant as shown by cytological data.

II. MATERIAL AND METHODS.

(i) Material, giving List of Species.

(ii) Methods.

II. MATERIAL & METHODS.

(i) MATERIAL[x]:

The following species were selected for examination: -

Species.	Exposure.
R. Albrechtii Max.	grown in the open.
R. arboreum, Smith	" " " "
R. barbatum, Wall	" " " "
R. campylocarpum, Hook f.	" " " "
R. cinnabarinum, Hook f.	" " " "
R. decorum, Franch	" " " "
R. Degronianum, Carrière	" " " "
R. discolor, Franch	" " " "
R. fulvum, Balf. f. & W. W. Smith	" " " "
R. galactinum, Balf. f.	" " " "
R. Hodgsonii, Hook f.	" " " "
R. Aucklandii X Thomsonii	" " " "
R. Schlippenbachii, Max	" " " "

R. /

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[x] The writer desires to express her gratitude to the Regius Keeper of The Royal Botanic Garden, Edinburgh, for permission to collect material from the plants in the Garden, and to various members of his Staff for help and assistance in so doing.

R. Souliei, Franch.	grown in the open.
R. Traillianum, Forrest & W.W.Smith.	" " " "
R. Ungernii, Tranto.	" " " "
R. Yunnanense, Franch.	" " " "
R. arboreum album	" under glass.
R. brachyanthum, Franch.	" " "
R. calophytum, Franch.	" " "
R. Keysii, Nutt.	" " "
R. megacalyx, Balf. f. & Ward.	" " "
R. Yunnanense, Franch	" " "

All the material for each species was obtained from one plant thus eliminating any genetical or physiological idiosyncrasy of the individual plant.

Collections of material were made at appropriate intervals, the material consisting of young buds each containing a whole inflorescence.

(ii) METHODS:

These buds were fixed - a variety of fixatives being used. Considerable difficulty was met with with all the fixatives tried as the buds do not readily sink in the liquid and resist penetration. These difficulties were overcome by making incisions in the bud or by dissecting out the individual flower-buds and immersing them. The use of an /

an exhaust pump on the vessel of fixative was found to be an absolute necessity even with dissected buds. The following fixatives were used: -

Chromo-acetic acid,
Acetic-alcohol,
Formalin-acetic-alcohol,
Formalin-alcohol,
Carnoy's Fluid.

Of these chromo-acetic acid (100 c.c. of 1% chromic acid in 50 c.c. of 1% acetic acid) proved easily the most satisfactory showing ready penetration and being convenient for use. Practically all the material reported on here was fixed in this medium as the chromo-acetic mixture demonstrated its suitability early in the preliminary work. Immersion in this fixative was continued for twenty-four hours, and, after washing in water, the buds were carried through a series of alcohols up to xylol by the standard method.

Embedding was in paraffin (melting point 50°C). Microtome sections (a Spencer Lens Microtome being used) 8μ in thickness, unless the contrary is stated, were cut. Haidenheims Iron-Alum Haematoxylin stain was relied upon for the main part of the work, though in some cases (where stated) the safranin-gentian-violet technique was used.

Fresh /

Fresh material was cut by hand from time to time, stained by Belling's Aceto-carmine method (1), and examined immediately. This method was restored to mainly to control times of making collections and proved of great value in arriving at an estimation of the state of the material before proceeding to preservation and embedding. Certain observations reported here were made on this fresh material and when such is the case, it is so stated in the text.

An examination of the slides was made by means of a Zeiss microscope and the drawings necessary were made with a Camera Lucida attachment.

III. SYSTEMATIC NOTES ON THE GENUS

AND

ENVIRONMENTAL CONDITIONS

OF THE

PLANTS STUDIED.

(i) Systematic Notes.

(ii) Table showing Habitats of Species studied.

(iii) Conditions under which these Species are grown in The Royal Botanic Garden, Edinburgh.

III. SYSTEMATIC NOTES ON GENUS & ENVIRONMENTAL CONDITIONS OF PLANTS STUDIED.

(i) SYSTEMATIC NOTES:

The genus Rhododendron besides being a very large one in respect to number of species, upwards of 1,100 species being known, is also very widely spread in the Northern Hemisphere, only one species, R. lochae, being found as far south as Australia. The largest centre of distribution is in the mountainous districts of S. W. China and the Eastern Himalayas, particularly in Sikkim and Bhutan, and in Indo-China, Malaya and New Guinea. Other species occur in the mountains of Central and S. Europe and Asia Minor. There is also a less important centre of distribution in N. America, while R. lapponicum is found within the Arctic Circle in Lapland. The Series Azalea occurs mainly in the temperate regions of the Northern Hemisphere - in Eastern Asia and N. America with an isolated species, R. luteum in the Black Sea Region. Although many of the species grow within the Tropics, yet owing to the altitude at which they grow, up to 15,000 - 16,000 feet (R. nivale) they may be looked upon as being temperate plants. In habit the members of the genus range from prostrate and /

and low-growing plants to large woody shrubs and trees; the majority of the species are evergreen but those of the Series Azalea are deciduous. The branch bearing an inflorescence, which is usually terminal in position, is continued by the formation of a bud in one of the upper leaf axils causing vigorous lateral branching. Large winter buds are formed covered with scale leaves which may be either coated with a viscid substance or covered with hairs; the larger and stouter buds contain inflorescences, the more slender ones being merely leaf-buds.

The fol~~lo~~wering period is a long one and may be extended to eight weeks; some species are spring and some are summer flowering. The flower is very uniform throughout the genus, the most notable feature being the absence of free pollen grains. The grains remain in tetrad formation and these tetrads are held together by slender viscid threads so that numerous tetrads are shed or drawn out from the anther simultaneously. The flowers are protandrous.

BOWER (7a) gives very full and detailed descrip:tions of the structure of the pollen tetrad and of the origin and subsequent development of the viscid threads and their relation to the pollen tetrads. Although he confines his attention mainly to one species, R. catawbiense, yet so uniform are the species in the details of their floral structure, it was /

was not thought necessary to carry out further investigations on these points in any of the other species.

(ii) TABLE SHOWING HABITATS OF SPECIES STUDIED:

Species.	Native Habitat.	Altitude.	Exposure in Royal Botanic Garden.
R. Albrechtii	Central & N. Japan.		In open in border protected N. & S. by high yew hedges.
R. arboreum	Temp. Himalayas, Kashmir to Bhutan, Khasia Mts, Ceylon.	5,000 - 10, 000 ft.	In open on slope facing south - also growing under glass.
R. arboreum album	Nepal, higher mountains	-	growing under glass.
R. barbatum	Summit of Tonglo, Sikkim Himalayas.	10,000 ft.	In open, protected N. & E. by larger shrubs overshaded by beech trees.
R. brachyanthum	Yunnan, Tsang-chau mount:ains.	9,000 ft.	growing under glass.
R. calophytum	W. Szechuan, at Mupin	7,000 - 10,000 ft.	" " "
R. campylocarpum	Sikkim-Himalayas.	11,000 - 14,000 ft.	In open protected N. & W. by Yew hedge, larger shrubby Rhododendrons growing near.

R./

Species.	Native Habitat.	Altitude.	Exposure in Royal Botanic Garden.
R. cinnabarinum	Sikkim-Himalayas	10,000 - 12,000 ft.	In open protected N. & W. by Yew hedge, larger shrubby Rhododendrons growing near.
R. decorum	Yunnan & Szechuan - wide distribution in Western China.	8,000 - 11,000 ft.	In open in border protected N. & S. by high Yew hedges.
R. Degronianum	Japan - a common species.		" " " "
R. discolor	Szechuan & Hupeh.	4,000 - 7,000 ft.	" " " "
R. fulvum	W. Yunnan	10,000 - 11.000 ft.	In open, protected by Yew Hedge N. & W. and with larger shrubby Rhododendrons growing near.
R. galactinum	Szechuan Province	9,000 - 10,000 ft.	" " " "
R. Hodgsonii	Himalayas, from Nepal to Bhutan.	10,000 - 12,000 ft.	In open, border protected N. & S. by high Yew Hedges.
R. Aucklandii X Thomsonii.			In open surrounded by large Rhododendrons and overshadowed by a specimen tree of Juglans regia.

R. /

Species.	Native Habitat.	Altitude.	Exposure in Royal Botanic Garden.
R. Keysii.	Mountains of Bhutan.	9,000 - 10,000 ft.	Growing under glass.
R. megacalyx.	N.E. Upper Burmah.	7,000 - 9,000 ft.	Growing under glass.
R. Schlippenbachii.	Korea, N.E. Manchuria, Central Japan.		In open inborder protected N. & S. by high Yew Hedges.
R. Souliei.*	from Szechuan to Yunnan, wide-spread.	9,000 - 11,000 ft.	In open surrounded by large Rhododendrons.
R. Traillianum	W. Lichrang Range, N.W. Yunnan.	11,000 - 12,000 ft.	In open protected by Yew Hedge N. & W. and with large shrubby Rhododendrons near.
R. Ungernii	Caucasus		In open, in border protected N. and S. by High Yew Hedges.
R. Yunnanense	Yunnan, at Houang-Li-pin.	9,000 ft.	In open, forming hedge with S. exposure, partially shaded by Beech trees - also growing under glass.

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* When originally described R. Souliei was listed as a wide-spread species with distribution as given in above Table, but in the Rhododendron Society Publication 1930, this distribution is noted as a mistake and the distribution given as "Western Szechuan: Tatsieulu; very distinct species of limited distribution".

(iii) CONDITIONS UNDER WHICH THESE SPECIES ARE GROWN IN THE ROYAL BOTANIC GARDEN, EDINBURGH.

The plants growing in the open are mainly on slopes with a southerly or south-easterly aspect, with large specimen trees of beech and other deciduous trees growing among them. The Rhododendrons are single shrubs, or in groups forming a hedge, or closely planted in borders protected on the north by a high hedge of Taxus baccata. Every year the soil is top-dressed with leaf-mould, beech and oak leaves particularly, and there is no cutting back of the shrubs.

In the glass-house the plants are favourably situated with regard to each other in the matter of light and air. The soil is similarly top-dressed every year. Watering is done fortnightly during the winter months and about thrice weekly in the summer with syringing with a hose on the intervening days. There is no methodical cutting back only as the plants tend to encroach on each other. The temperature varies with the outside temperature, showing an average temperature of 40°F, but in no case is it allowed to fall below a minimum of 32°F, the heat being turned on at night if there appears to be a possibility of frost; so that the glass-house plants are not subjected to freezing temperatures.

IV. THE NORMAL CYCLE OF FORMATION
OF THE
SEXUAL TISSUES, -
e.g., R. YUNNANENSE, Franch.

(i) History of Plant and Material.
(ii) Formation of Male Tissues.
(iii) Formation of Female Tissues.
(iv) Table showing corresponding Stages in Development in Male & Female Tissues.
(v) Distribution of Tannin in the Flower and the Vascular Supply.
(vi) Summary.

IV. NORMAL CYCLE OF FORMATION OF THE SEXUAL TISSUES, e.g., R. YUNNANENSE, Franch.

(i) HISTORY OF PLANT AND MATERIAL:

The majority of plants, as already stated, have a considerable interval of time elapsing between the initiation of the sporogenous tissue in the anther and the first sign of ovule formation in the gynoecium. The stages of these processes seem to be very uniform throughout a large number of species and so in only one, R. Yunnanense, will these stages be described in detail, this species being taken as a standard. This species was first described by FRANCHET (19) in 1886 from material collected at Houang-Li-pin in Yunnan. The plant growing in the open in the Royal Botanic Garden, Edinburgh, from which the material for this study was taken, was raised from seed in the Botanic Garden, the seed having been sent from Yunnan.

Flower-bud material of this species was collected from plants both growing in the open and under glass; the complete cycle was obtained from the outside material, the glass-house plant being used as a check.

In 1929 this species flowered under glass during the month ending May 4th and in the open during /

during the month ending June 1st. In 1930 Yunnanense flowered under glass during May and in the open during the month ending June 7th. In 1931 the flowering was recorded under glass during May and in the open for the month ending June 6th. Thus in the period during which this study was made, the glass-house plant flowered a little earlier than the one outside. Collections of flower-buds were made during the years 1930, 1931 as given below:

R. Yunnanense (Plant outside):

Collection of flower-buds	22/ 8/30	preserved
" " " "	1/ 9/30	"
Fresh Material examined	19/ 1/31	
" " "	2/ 3/31	
Collection of flower-buds	2/ 3/31	"
" " "	17/ 3/31	"
" " "	27/ 3/31	"
" " "	3/ 4/31	"
Collection of gynoecia only	4/ 5/31	"
" " " from open flowers	8/ 5/31	"
" " flower-buds	15/ 7/31	"
" " "	17/ 7/31	"
" " "	20/ 7/31	"
" " "	23/ 7/31	"
" " "	6/ 8/31	"
" " "	9/ 9/31	"
Fresh Material examined	16/10/31	"
Collection of flower-buds	21/10/31	"
" " "	10/11/31	"

(ii) FORMATION OF MALE TISSUES:

Before proceeding with a detailed description the writer would point out that the term "male tissues" is used to include the different parts of the male organ, the filament and anther of the stamen /

stamen as well as the sporogenous tissue contained in the pollen-sacs; similarly the term "female tissues" includes the whole gynoecium as well as the placentae and ovules, the term "female sporogenous tissue" being used to indicate the ovules alone.

The formation of the sexual tissues may be taken separately. In all cases the figures of the anthers and loculi of corresponding stages were taken from the same flower-bud.

The first collection of flower-buds was made on August 22nd, 1930, about six weeks after the first record of flowering for that year. On sectioning it was found that the stamens were fully differentiated into filaments and anthers, there being a single vascular bundle running up the centre of the filament and continuing into the anthers. Each bundle was partially surrounded by cells containing tannin forming an incomplete sheath. The initial sporogenous tissue was clearly visible in each of the four pollen-sacs of the anther in the form of a small group of cells, each cell slightly larger in size than those of the adjacent tissue and containing a large, deeply-staining, granular nucleus - (Fig. I).

The material collected from the same plant ten days later, Sept. 1st., showed a much larger area of sporogenous /

sporogenous tissue, the cells of which had the same characteristics as previously, while the cells of the outer wall of the anthers had much thicker cell-walls than in the previous collection. (Fig. II.).

Fresh material was examined on Jan. 19th, 1931. The pollen was well-developed, lying loose in the pollen-sacs in tetrads. Many of the inflorescence buds at this date appeared to have suffered damage, at least superficially, from frost, but the flower-buds contained within them, on examination showed that pollen-tetrads were fully developed.

The next collection of material was made on March 2nd. Fully formed pollen-tetrads were present lying freely in the cavities of the pollen-sacs and the tapetal layers were partially collapsed. Here and there the cells of the tapetal layers showed contents, but the cell-walls of the majority of the tapetal cells and also those forming the wall of the pollen-sacs exhibited considerable crumpling. The cells of the outer wall of the anthers showed thickening cell-walls as mentioned previously, and also abundance of tannin (similar to condition shown in Fig. IV.).

Fresh material of this date (March 2nd.) was also examined, when it was found that in some of the inflorescences the central flower-buds were brown and withered in appearance, while the pollen-tetrads which /

which had been formed were also withered. This arrested development was probably due to the fact that in the preceding month, February, there had been a severe and prolonged snowstorm following on rather milder weather. This would, no doubt, taken together with the frosting mentioned earlier, constitute a check on further growth, while the pollen formed would tend to shrivel.

In all subsequent material collected, pollen was present as fully formed tetrads and was efficient in its action (Figs. III. and IV.). Although the actual reduction divisions in the pollen-mother-cells were not observed in this species, it would seem that the process must have taken place in the early autumn, probably in September prior to the setting in of the winter dormant period for the plant.

A collection of fresh material gathered Oct. 16th, 1931 showed pollen-tetrads formed and the tapetal layer intact.

The process of development in the glass-house specimen followed similar lines, but was slightly in advance of the outside plant.

(iii) FORMATION OF FEMALE TISSUES:

The sections made from the earliest collection of material, 22nd. Aug. 1930, showed but the beginning of the locular spaces into each of which projected a mass of tissue from the central tissue of the gynoecium. This projecting mass of tissue was the undifferentiated placentae and was partially divided into two by a split in continuation of the locular space. The placental tissue consisted of a single epidermal layer of regular cells enclosing a mass of rather larger cells more irregular in form and having large granular nuclei. The walls of the loculi as far as they had developed, were composed of a layer of large cells with a thickening of their walls on the free surface. (Fig. V.).

In the next collection, that of Sept. 1st. 1930, the same condition was seen in the gynoecium as in the previous material, there being a larger number of cells in the placental area with the locular space slightly more enlarged. This difference in size was possibly due to a difference in the size of the bud examined. However, essentially, the gynoecia of these two collections were at the same stage of development. (Fig. VI.).

The examination of the material collected 2nd. March /

March 1931, revealed a further stage in the development. The locular space had extended in towards the centre, while the placental masses had much increased in size, the separation of the two masses in each loculus being practically complete. The placental mass also showed more differentiation - unequal multiplication of cells had taken place so that the placenta appeared to be contracted in parts, particularly towards the outer portion of the loculus with the result that small groups of cells surrounded with epidermal cells tended to become cut off from the main mass of placental tissue. These small groups proved to be the initiation of ovules, in some of the groups an initial archesporial cell being indicated. (Fig. VII.). The locular wall was well marked out, the cells showing a thickening of all walls.

In the material collected 17th March, the contraction of the placentae was more evident so that practically all the placental tissue was divided into club-shaped masses attached to a central rod of tissue. (Fig. VIII.). Many of these immature ovules showed archesporial cells situated immediately underlying the epidermal layer, and were two to three times as large as the adjacent cells. The nucleus of such an archesporial cell was very large and granular, possessing a large, deeply-staining nucleolus (Fig. /

(Fig. IX.).

In the material collected ten days later, 27th. March, a further stage was seen, the loculi were enlarged and the ovules had increased in size, all the ovular tissue having now been organised. The ovule showed a thickening of the walls of the epidermal cells and an increase of cell growth a short distance behind the archesporial cell, this extra growth extending outwards and tending to overarch the archesporial cell thus marking the commencements of the comparatively massive integument. (Fig. XI.).
The archesporial cell had greatly enlarged as had also its nucleus. The granular portion of the nucleus and also the nucleolus took the stain very deeply, while the outlines of both the nucleolus and the nucleus itself tended to become irregular.

In some cases the archesporial cells appeared to have undergone division, sister-cells, which later went to form the nucellus, being evident, so that the original archesporial cell in such a case had become the mega-spore-mother-cell. (Fig. XI).

A week later - 3rd. April, material was again collected. The gynoecia appeared to be in a similar condition to that seen in the preceding collection with similar mega-spore-mother-cells showing. Drawings were therefore not made of this material.

On /

On 4th May the plant was partially in flower, but gynoecia from as yet unopened flowers were dissected out and treated. Drawings were made of the ovules in section. These revealed a thick-walled epidermal layer of cells surrounding nucellar tissue in which the embryo-sac was embedded. The embryo-sac showed densely granular contents and a large nucleus which contained a large nucleolus and, in addition to minute granules, some large granules which stained deeply (Figs. XII, XIII).

Gynoecia from fully opened flowers were collected on 8th May and treated. On sectioning the ovules were seen to contain embryos consisting of a single cell connected to the surrounding tissue by 3 or 4 suspensor cells. The nucleus and nucleolus had much decreased in size in comparison with the size of the cell (Fig. XIV.).

In material collected 16th Oct. 1931, the placental tissue was well defined and ovular constrictions initiated. The condition of the male tissues at this date has already been noted. This material consisted of flower-buds for 1932 flowering. It would thus appear that the stages seen in this material were the winter condition of these tissues.

A comparison with the gynoecia of the glass-house plant gave a similar course of development but /

but, as in the case of the development of the male tissues, the development was a little earlier in taking place. The material collected from the glass-house plant, 12th Mar. 1931, showed the gynoecium to be in a condition similar to that found in the outside plant on 17th March 1931; while the gynoecia from the glass-house plant, collected 17th March 1931, were in a condition approaching that seen in the outside plant on 27th March, 1931. (Figs. X, XI.)

(iv) TABLE SHOWING CORRESPONDING STAGES OF DEVELOPMENT IN MALE & FEMALE TISSUES.

	Date of Collection	Male Tissues.	Female Tissues.
R. Yunnanense Plant grown in the Open.	22/8/30.	Fully organised stamens; small amount of sporogenous tissue present. (Fig. 1.).	Loculi defined; placental tissue indicated. (Fig. V.)
	1/9/30.	Larger area of sporogenous tissue present. (Fig. II).	Similar to condition seen in 22/8/30 but larger. (Fig. VI).
	19/1/31 (fresh material).	Pollen-tetrads well developed and lying free.	Not noted.
	2/3/31 (fresh material).	Pollen-tetrads of central buds of inflorescence withered.	Not noted.
	2/3/31	Pollen-tetrads lying free; tapetum partially collapsed. (cf. Fig. IV.).	Loculi larger; placental tissue showing constrictions. (Fig. VII).
	17/3/31	Similar condition to that seen in material 2/3/31 (Figs.III & IV).	Placental constructions more marked; ovule rudiments larger. (Fig. VIII & IX).
	27/3/31		

	Date of Collection	Male Tissues.	Female Tissues.
R.Yunnanense	27/3/31	Similar condition to that seen in material 2/3/31.	Loculi larger; all ovular tissue organised; initiation of integument (Fig. XI).
	3/4/31	" " " "	Similar to condition in 27/3/31.
	4/5/31 (Plant partially in flower)	" " " "	Ovules fully formed and showing embryo sacs. (Figs. XII & XIII).
	8/5/31 (Fully opened flowers)	" " " "	Ovules showing young embryos. (Fig. XIV.).
Plant grown under glass	12/3/31		Condition similar to that seen in material 17/3/31 from plant in open (See above) (cf.Figs. VIII & IX).
	17/3/31	Pollen-tetrads lying free, tapetum collapsed.	Ovules more organised than in 17/3/31 (Open plant); condition approaching that of 27/3/31 for open plant (See above). (cf. Figs. X. & XI.)

(V) DISTRIBUTION OF TANNIN & VASCULAR SUPPLY.

The distribution of the cells containing tannin in the sexual tissues proved of some interest. In the flower-buds of the earliest collection, August 22nd. 1930, the petals showed two layers of tannin-containing cells on both upper and lower surfaces. In the stamens the tannin appeared in a few cells forming a partial sheath round the vascular tissue of the filaments and anthers, and also appeared in the cells of both the epidermal layers of the stamens. In the female tissues of this date of collection the tannin-containing cells were more or less irregular in distribution, forming a definite layer around the ovary and defining the wall of the loculi, as well as being situated singly here and there throughout the general mass of tissue in the gynoecium. The vascular bundles of the gynoecium did not show any indication of a tannin sheath. (Fig. XV.A.)

In spring the mature stamens were found to contain many more tannin-cells these forming a complete sheath of several layers around the vascular tissue; all the cells of the epidermal and sub-epidermal layers also contained tannin. In the gynoecia of material collected in March 1931, the tannin-containing cells formed more definite layers than previously /

previously. Two to three layers of such cells formed the locular walls, while a similar band of cells also formed the wall of the ovary. In the tissue lying between the locular and ovarian walls were numerous groups of tannin-containing cells. The vascular bundles, however, did not show any definite sheath of tannin-cells. (Fig. XV. B.).

In the gynoecia of the mature flowers, the area of tannin-containing cells had increased considerably. The band of tannin-cells forming the locular wall was continued into the placental tissue as a smaller band lying below the surface layers of cells; this tannin tissue also extended so as to enclose almost completely the vascular strands of the placenta. The mature ovules also showed tannin deposited in the cells of the integument. The band of cells forming the outer wall of the ovary had increased in width, but the cells forming it did not exhibit such dense tannin contents as the similar cells of the locular walls. The tannin-cells lying intermediate between these two walls and hitherto in isolated groups, had now become connected into a continuous band of varying width which also enclosed the vascular bundles lying in this region. (Fig. XV.C.).

(VI) /

(VI) SUMMARY:

In summing up these processes of development briefly it is noted that: -

(a) The flower-buds of any one flowering season are initiated about six weeks to two months after the preceding flowering season has ended.

(b) The tissues of the androecium and gynoecium are laid down simultaneously.

(c) The male tissues in the bud develop more rapidly than the female tissues - stamens being found fully formed and showing sporogenous tissue six months before there is any sign of placental differentiation.

(d) Pollen-tetrads are fully formed by the end of the autumn preceding flowering and ovular constrictions are showing in the placental tissue at that date.

(e) The flower-buds pass through a dormant winter period when development is in abeyance.

(f) The female sporogenous tissue begins its development again in early spring - "ovular constrictions" stage being the winter condition.

(g) Tannin is deposited in the cells at a very early stage and increases in amount as development proceeds - finally ensheathing the vascular tissue.

(h) The vascular tissue, in both male and female tissues, is at first without a sheath but shows a complete sheath of tannin-containing cells when the gemetes are fully formed. In the ovary the vascular tissue increases in amount as development proceeds.

V. NOTES on CYCLES
of
OTHER SPECIES.

V. NOTES ON CYCLES OF THE SPECIES.

(I) INTRODUCTION:

In order to ascertain whether, as between - species and species, there was any deviation from the normal cycle of development of the sexual tissues described above for R. Yunnanense, collections of material from representative species, growing both in the open and under glass, were made during the course of the year. For the species from which such collections of material were made see the list given in Section II., Material and Methods.

It was found that the course of development was very uniform throughout, the variation being in the time interval between the conclusion of the flowering period and the initiation of the parts of the flower for the succeeding season.

A. PLANTS GROWN IN THE OPEN:

(See Figs. XVI - XXX).

(II) TABLE SHOWING VARIATIONS:-

In the Table below the variation in this time interval and the corresponding stages of development for species grown in the open, are given: -

Table showing Variations in Time Interval and Stages of Development:

Species.	Exposure	Flowering recorded 1930	Flowering recorded 1931	First Collection of Material.	Condition of Sexual Tissues at First Collection. Androecium	Condition of Sexual Tissues at First Collection. Gynoecium
R. arboreum	open	Apl-May	1st.Week May	7th July 1930	Stamens organised; no sporogenous tissue evident.	Gynoecium organised; locular splitting just initiated.
R. barbatum	"	March	March	7th July 1930	Large amount of sporogenous tissue present.	Placental tissue well defined.
R. campylocarpum	"	1st. week May	1st.Week May	5th July 1930	Large amount of sporogenous tissue present; tapetal layer completely formed.	Placental tissue well defined.
R.Degronianum	"	1st.week March	March	7th July 1930	Pollen-mother-cells present	Placental tissue showing initiation of ovular constrictions.

Species.	Exposure	Flowering recorded		First Collection of Material.	Condition of Sexual Tissues at First Collection.	
		1930	1931		Androecium	Gynoecium.
R. fulvum	Open	2nd week April	2nd Week April	5th July, 1930.	Sporogenous tissue evident.	Placental tissue initiated
R. galactinum	"	1st week June	May - June	9th July 1930.	Sporogenous tissue evident.	Placental tissue well defined.
R. Schlippenbachii.	"	1st week June	1st. week April	27th Mar. 1931.	Pollen-tetrads present and lying free.	Fully formed ovules present showing embryo-sacs.
R. Souliei	"	1st week June	May-June	7th July, 1930.	Pollen-tetrads and collapsed tissue in some pollen sacs; in others pollen-mother-cells and normal tapetum.	Ovule rudiments large but ovules not fully constricted off from placental tissue.

(III) ANALYSIS OF FOREGOING TABLE:

On an analysis of this Table for plants growing in the open, it is found that: -

(a) R. Degronianum is the earliest to flower of the species examined - flowering in early spring, the first week in March - and shows pollen-mother-cells and ovule initials four months after that flowering (first week in July).

R. barbatum, the next earliest flowering species (about a fortnight later than R. Degronianum) exhibits sporogenous tissue in the stamens and well defined placental tissue in the gynoecium by the first week in July; but does not show a condition approximating to that seen in Degronianum at the first week in July, until the middle of August.

(b) The latest flowering species in 1930, R.galactinum, Schlippenbachii and Souliei, of those examined, gave evidence of a very rapid growth of the floral organs. In galactinum the male sporogenous tissue and the placental tissue are well defined within six weeks of the end of the flowering period. (Figs. XXII, XXIV.). In Souliei pollen-tetrads and large ovule rudiments are found six weeks after flowering (Fig. XXVIII.). In the latter case it may be suggested that the material examined was but belated buds of the 1930 flowering /

flowering period. But if that were the case then the ovular de~~part~~velopment would have to have been very rapid for the open flower to contain fully developed ovules. This fact can be better appreciated if the third species, <u>Schlippenbachii</u> is considered. Here the week prior to flowering shows the ovules fully formed and embryo-sacs present. This is also the case with <u>Yunnanense</u>, an April flowering species. There were large ovular rudiments evident in <u>galactinum</u> by the middle of August (Fig. XXV.), similar to those seen in <u>Souliei</u> at the first week in July, (Fig.XXVIII), and that within two months of flowering. By the middle of March 1931, the condition in the gynoecia of <u>galactinum</u> was well-defined ovules present but no embryo-sacs (Fig. XXVI.). Therefore the ovular development from the middle of August 1930 to the middle of March 1931, was very slow.

(c) If a definite stage in development is taken as, for example, "placental tissue well defined" - then, for the species given in the above Table, the following intervals of time elapse between flowering and the attainment of that definite stage : -

	<u>Flowering.</u>	<u>Placental Tissue w. def.</u>
<u>arboreum</u>	1st. week May, 1930.	1st. Sept., 1930. (Fig. XIX.).
<u>barbatum</u>	middle March "	7th July, 1930.

campylocarpum /

	Flowering.	Placental Tissue w. def.
campylocarpum	1st week May 1930	5th July 1930.
Degronianum	1st week Mar. "	(Prior to (7th July "
fulvum	2nd week April "	5th July "
galactinum	1st week June "	9th July "
Souliei	" " "	(Prior to (7th July "

or, expressing the above data more generally, there is an interval between flowering and the attainment of a definite stage, of -

4 months in the case of arboreum
3- 4 " " " " " barbatum
3 - 4 " " " " " Degronianum
2 - 3 " " " " " fulvum
2 " " " " " campylocarpum
1- 2 " " " " " galactinum
1 month " " " " Souliei.

Similarly if comparisons are made using a definite stage in the development of the androecium, as, for example, the stage of "sporogenous tissue evident", then the time interval elapsing before the attainment of that stage by any of the species is -

	Flowering.	Sporogenous Tissue evident.
arboreum	1st.week May 1930	1st. Sept. 1930 (Fig. XVII).
barbatum /	Middle March "	7th. July. 1930.

	Flowering	Sporogenous Tissue evident.
campylocarpum	1st.week May 1930	5th July, 1930.
Degronianum	1st week Mar. "	(Prior to (7th July "
fulvum	2nd week Apl. "	5th July "
galactinum	1st week June "	9th July "
Souliei	1st week June "	(Prior to (7th July "

Thus it will be seen that the time required to attain this definite stage in development of the male tissues is the same as that taken to attain the corresponding stage of female development for that particular species.

(d) From the foregoing facts it is seen that a definite stage of development of the female tissues corresponds to a definite stage of development of the male tissues in the above species. When the male sporogenous tissue is definitely present, in greater or less amount, as in barbatum, campylocarpum, fulvum, galactinum, then the corresponding stage in the female tissues is the presence of definite placental tissue but without sign of ovular constrictions. In the case of Yunnanense these were also seen to be corresponding stages. Again, when pollen-mother-cells are present then, in the female tissues, ovular rudiments are evident, e.g. Degronianum/

Degronianum. The attainment of the pollen-tetrad stage is the final one in the male series and persists from early autumn until spring. Thus this stage corresponds to all the final stages of female development. In galactinum at the beginning of September, pollen-tetrads were present and very large ovular rudiments; similar stages corresponded in Souliei earlier in the year, the male tissues having been found then in a transitional state (see Table). The growth of the female tissues after the attainment of the pollen-tetrad stage by the male tissues is very slow - in Yunnanense the pollen-tetrad stage has just been reached (the tapetal cells being still normal and showing no signs of collapse) when the ovular rudiments are well-defined; (Figs. III, VII); the spring material shows the ovular rudiments larger but the ovules not fully constricted off; while the pollen-tetrads are normal but the tapetum has degenerated (Figs. IV, VIII).

This point will be discussed at greater length in a later section of this paper.

B. PLANTS GROWN UNDER GLASS. (See Figs. XXXI-XLIII).

(IV). TABLE SHOWING VARIATIONS:

With regard to the species grown under glass, a Table similar to the preceding one for species grown in the open, was compiled.

IV. TABLE SHOWING VARIATIONS IN TIME INTERVAL AND STAGES OF DEVELOPMENT:

Species.	Exposure.	Flowering recorded		First Collection of Material.	Condition of Sexual Tissues at First Collection.	
		1930	1931		Androecium	Gynoecium.
R.arboreum album	Under glass	March	Feb.March	8th July, 1930.	Small amount of sporogenous tissue evident (Fig. XXXI.)	Placental tissue initiated. (Fig.XXXIII).
R.brachyanthum	" "	June - July	July	2nd.March, 1931 (prior to flowering 1931)	pollen-tetrads fully formed; tapetum still evident; in some cases nucleus of pollen-grain divided.	Ovular constrictions well marked.
				17th Sept. 1931 (buds for 1932 flowering)	Sporogenous tissue evident.	Placental tissue initiated.

R./

Table Continued:

Species	Exposure	Flowering recorded		First Collection of Material.	Condition of Sexual Tissues at First Collection.	
		1930	1931		Androecium	Gynoecium.
R. calophytum	Under glass	March	Feb.March	16th. July, 1930.	Stamens organised; no sporogenous tissue evident.	Locular splitting just initiated - septa of carpels not completely united.
R. megacalyx	" "	June		2nd.April, 1931 (prior to flowering, 1931).	Pollen-tetrads fully formed and lying free.	Ovules delimited - embryo-sacs evident.

V. ANALYSIS OF TABLE COMPILED FOR THE GLASS-HOUSE SPECIES.

As in the species grown outside, it was found with reference to the glass-house species that the time required to attain to a certain definite stage of development varied with the species. The stages given above "sporogenous tissue evident" and "placental tissue well defined", were attained in the case of R. brachyanthum two months after flowering while the time required by R.arboreum album (a colour form of R. arboreum) was about four months. As seen previously, certain stages in the male and female tissues corresponded and this correspondence was the same in all the species. This same general rule was found to hold for the glass-house species too.

It was also noticed with regard to R.brachyanthum that, after flowering (1931) there followed a short period of vegetative growth, despite the fact that vegetative growth had been made in the spring prior to flowering. This condition, it was ascertained was not unusual in plants under cultivation, especially when growing under glass-house conditions, and might also be found in cultivated plants growing in the open. This extra vegetative growth did not apparently have any effect on the development of the sexual tissues as, in this species, flower-buds for the /

the next season's flowering (1932) were formed as usual soon after the 1931 flowering had ceased.

In their native habitats the period of vegetative growth of members of the genus occurs in the spring and may proceed simultaneously with the flowering as in the spring-flowering species, or be prior to flowering as in the case of the summer-flowering species. In the case of R.Schlippenbachii a deciduous species, the leaves and flowers appear simultaneously.

(VI) SUMMARY:

A summary of the facts brought forward in this section may be made briefly: -

a) A definite interval of time elapses between the flowering period of one year and the attainment of any stage in the development of the floral organs for the succeeding year. This interval is longer in the case of the spring-flowering species than in the summer-flowering species.

b) The rate of floral development up to the onset of winter, varies according to the season of the flowering period of the plants. Spring-flowering species show a comparatively slow rate of new floral growth compared with the more rapid rate of the summer-flowering species.

c) /

c) During the winter season, the male tissues undergo a period of more or less complete dormancy, while the female tissues continue to develop but very slowly.

d) There is a correlation between the rates of development of the male and female tissues - certain definite stages corresponding in all species.

VI. ABNORMAL CYCLE

of

FORMATION OF SEXUAL TISSUES.

e.g., R. Keysii, Nutt.

VI. ABNORMAL CYCLE OF FORMATION OF THE SEXUAL TISSUES: e.g., R. Keysii, Nutt.

(i) INTRODUCTION AND HISTORY:

During the course of examination of the various plants one species, R. Keysii, was found to differ markedly from the normal cycle of sexual tissue development as given in detail for R. Yunnanense.

The two plants of this species in the Gardens, both growing under glass, were therefore kept under observation. Both these plants had been reared in England and acquired by the Regius Keeper of the Gardens at an early age. They were both planted under glass at a short distance from each other in 1920. They differed in that the one flowered every season and had never been cut back, while the other had never flowered at all. The flower-bearing plant had always set seed but it had never been ascertained whether the seed was fertile.

(ii) MATERIAL:

The position of the inflorescence-buds on the plant was of some interest. In April 1931, terminal buds were examined and appeared to be purely vegetative; on closer examination minute buds were discovered in the axils of the outer leaves of these terminal buds proving themselves, on further investigation /

investigation, to be inflorescence buds. As the terminal leafy bud unfolded, these lateral inflorescence-buds became more evident and yielded minute flower-buds on dissection. Examination showed that the terminal leafy bud was the new vegetative spring growth for 1931, while the lateral buds were borne on the end of the preceding autumn wood. Hence it was concluded that the flower-buds and the spring vegetative growth developed simultaneously.

A comparison was made with the companion plant which had never flowered. The terminal vegetative buds were well developed and were further unfolded than the corresponding ones on the flowering plant at any time of comparison. The lateral buds were, however, definitely absent. The presence of the developing flower buds, although not entirely checking vegetative growth hindered it, so that of the two plants, grown under similar conditions, the one showed stronger and more rapid vegetative growth than the other.

The records of the flowering of this plant are:

1930 - Flowering commenced during month ending July 5th.
1931 - Flowering commenced during month ending June 27th., an inflorescence being open June 10th, and continued during July and the greater part of August. All the inflorescences were withered and the fruit maturing at the end of August.

Certain /

Certain of the inflorescence-buds, it was noticed, contained two kinds of flower-buds differing in external appearance. The central bud in these inflorescences, was very large in comparison with the others and topped the central core of tissue of the inflorescence. Owing to its position it appeared to be regularly symmetrical and did not show any unequal lateral development as the others did through their being appressed laterally against the central core. The central bud, also, did not show the usual two hairy sepals. In other inflor:escences this large central bud did not appear.

Collections of material were made as given in the following list: -

R. KEYSII.

Collection of flower-buds	2/ 4/31	preserved.
" " "	16/ 4/31	"
" " "	17/ 4/31	"
" " "	18/ 4/31	"
" " "	21/ 4/31	"
" " "	23/ 4/31	"
" " "	6/ 5/31	"
examination of fresh material ..	14/ 5/31	"
Collection of flower-buds	27/ 5/31	"
" " "	4/6 /31	"
" " " open flowers	and 10/ 6/31	"

(iii) DEVELOPMENT OF MALE TISSUES:

As in previous sections of this paper all figures /

figures of anthers and loculi at corresponding stages were made from the same bud.

The first collection of flower-buds, 2nd April, 1931, showed the different parts of the flower fully differentiated; ten stamens were present organised into filaments and anthers. The anthers, however, did not show any indication of sporogenous tissue.

The collections made a fortnight later, 16th. April, revealed the presence of a very definite area of sporogenous tissue surrounded by a layer of cells which marked the tapetal layer. The sporogenous tissue consisted of a number of large irregularly-shaped cells fitting closely into each other with cellwalls thicker than those present in the surrounding cells and having very abundant contents of a granular nature with large nuclei and nucleoli; in some cases two nucleoli appeared to be present in the one nucleus. The tapetal layer consisted of a single row of cells smaller in size than the sporogenous cells but larger and with more abundant contents than the ordinary parenchymatous cells of the anther. (Fig. XLIV.).

The collections made the next day, 17th April, showed a very great advance in the state of the sporogenous tissue. The buds sectioned were in all probability more than a day older in period of development /

development than the preceding ones although there was no means of ascertaining this. The sporogenous tissue had increased greatly in area and the cells had been in very active division. The results of these divisions were that many of the cells showed two complete nuclei each with a well defined nucleolus; others contained nuclei in which two nucleoli appeared, while others again showed a single large nucleus taking up almost the whole of the cell. (Figs. XLV., XLVI.). Although numerous mitotic figures were seen at this stage, it proved impossible to obtain counts of the chromosomes owing to their small size even under high magnification (x 2500). There was evidence, however, that the nuclei were undergoing reduction division. The tapetal cells formed a single layer, in a few places a double layer, around the sporogenous tissue. Active division was also seen in some of these cells, but they did not differ in character from the tapetal cells of the preceding collection.

The collection made the next day, 18th. April, on examination, revealed the same conditions as in the preceding day's material. The nuclei of the sporogenous cells, however, did not show active division and the sporogenous cells were uninucleate. Probably the time of day at which the collections were /

were made accounted for this difference. No drawings were made of this collection.

A very different phase was in evidence in the material collected on 21st. April. The pollen-sacs did not all show equal development; in some anthers only two were developed, the other two being small in size and the cells crushed in appearance with little tapetal and little sporogenous tissues. In the developed pollen-sacs the tapetal cells had increased greatly in size and showed numerous nuclei, two being present in many cells and in some cases three nuclei were present in a single cell. The central sporogenous tissue was small in comparison and appeared to be disintegrating. The cell-walls were thin and indistinct while the nuclei had disappeared in some cases, and in others had broken up into various sized granules more or less completely surrounded by the nuclear membrane; no definite nucleolus was observed. (Fig. XLVII.).

The material of 21st. April was apparently an abnormal condition, for that collected two days later, 23rd. April, showed fully-formed tetrads lying free in some of the pollen sacs, with a definite single-layered tapetum surrounding the cavity of the pollen sac. These tapetal cells were uninucleate. It was found, however, that the /

the pollen-sacs of different anthers varied in degree of development. Some had developed normally and appeared as described (Fig. XLVIII); others were similar to the condition seen in the drawing illustrating the abnormal condition with, however, the sporogenous tissue not quite so indefinite, the outline of the pollen-tetrads, being slightly better defined.

No drawings were made of material collected, 6th May, as the condition was similar to that seen in material of 21st. April.

An examination of fresh material was made on 14th. May. The central, large, symmetrical bud of the inflorescence, to which reference has already been made, showed free pollen-tetrads. In a lateral bud from the same inflorescence the stamens were only about half the size of those seen in the central bud, but contained large quantities of well developed pollen-tetrads. The two flowers were therefore at the same stage of development in regard to the male tissues but differed in size.

In the examination of material collected 27th. May, pollen-tetrads were seen lying free in the pollen-sac, a condition similar to that seen in the previous collection, but the tapetal cells were partially collapsed.

In /

In all succeeding collections a similar condition was seen in the anthers.

Pollen tetrads from a fully-opened fresh flower and also from an unopened flower, collected 13th. July, were placed in a 10% sugar solution. The pollen was examined two days later and several drawings made. (Fig. XLIX). It was observed that there was a quantity of bad pollen and that the germination was scanty. Of the four grains of a tetrad it was observed that only one grain germinated where germination had taken place. A further quantity of pollen from several opened flowers, collected 16th July, was also placed in a 10% sugar solution. The solution was examined four days later. Underdeveloped grains were noticed in many of the tetrads, and, as previously, germination was scanty. Counts were made and it was found that roughly the germination percentage was 19%.

This poor germination was hardly to be wondered at, since the development of the male tissues had been accomplished in a much shorter time than is usual for the genus and the winter dormant period had been practically obliterated. This abnormal cycle would therefore tend to produce some abnormality in the tissues. This point will be dealt with more fully in later sections of this paper.

(IV). /

(IV) DEVELOPMENT OF FEMALE TISSUES:

The first collection of material, 2nd. April 1931, consisted of very minute buds. The degree of development of the androecium has already been mentioned; but the ovary, although formed, consisted of a uniform mass of cells with no indication of locular spaces. In the style the tissue was hollow in the centre from which small splits extended outwards into the cellular tissue giving a stellate appearance in transverse section.

The second collection, 16th April, showed the loculi definitely defined and the placental tissue indicated. The locular wall was of two layers of cells as was also the ovary wall. The separation of the two placentae of any loculus did not extend very far in towards the centre of the ovary. (Fig. LII).

The material of the succeeding collection, 17th April, revealed the same condition in the gynoecium, although, as already stated in describing the development of the male tissues, the inflorescence bud was probably more than one day older in period of development than the preceding material. Thus it would appear that while the development of the male and female tissues proceeds simultaneously, they develop at different rates. (Fig. LIII).

The same condition as that of the previous day appeared in the ovary of the next collection, 18th. /

18th April, so no drawings were made.

A very marked advance was seen in the female development in the collection of 21st. April. The loculi were fully formed, the separation of the placentae was complete, while the placental tissue was showing definite constrictions marking the initiation of the ovules. The cells of the placent:al tissue in many cases showed nuclei containing two nucleoli. (Fig. LIV).

No drawings were made of the ovaries of the next collections, 23rd. April, and 6th May, as the con:dition of the former appeared to be exactly similar to the preceding one and the latter was similar to that of the 17th April.

From the fresh material collected and examined 14th May, it was found that in both the central, large symmetrical flower and the lat\eral small flowers ovules were well developed, especially in the latter case where they were prēsent in very large numbers.

The material collected 27th May, showed very little advance on that collected 21st. April. The constrictions marking out the ovules in the placent:al tissue were more marked and the ovule rudiments larger. (Fig. LV.).

The ovarian tissue of the material collected 4th June was badly prepared and so examination was impossible /

impossible.

The last collection of material was made on 10th. June, when some of the inflorescence-buds on the plant were open. The matérial, however, was taken from unopened buds and showed the ovules definitely formed. Free-hand sections were also made of gynoecia from fresh opened and unopened flowers on this date. The conditions appeared to be similar in both flowers, the ovules being definitely formed and embryo-sacs evident (Fig. LVI).

(V). REGULAR AND IRREGULAR FLOWERS:-

The preserved material, collected on 23rd. April consisted of the two kinds of flower-buds found in the same inflorescence and already mentioned. In the smaller, laterally compressed flowers there were five petals present, free at the tips but forming a tube round the androecium; ten stamens were present the condition of the anthers being as already described (p. 53); the ovary consisted of five loculi in which the ovules were at a similar stage to that seen in the material collected 21st. April (p. 57).

In the abnormal flower from this inflorescence there appeared to be five petals present but they were joined the whole of their length except at one side where the corolla tube was split down to the base /

base. Nine stamens only were present, the condition in all the anthers being similar to that seen in some of the anthers of the normal flower-bud of the collection of this date, having fully-formed pollen-tetrads lying free and a single-layered tapetum. The nuclei of the tapetal cells were, however, somewhat different from those in the more normal flower-bud in that they appeared to be disintegrating. (Fig. L.). In the ovary, which was very hairy, there were five loculi as in that of the corresponding more normal flower. The ovules, however, were much further advanced than in the smaller bud, in that they were definitely formed, being of the typical shape and stalked, while in the majority of the ovules embryo-sacs were present and the ordinary cells appeared to be in active division. (Fig. LVII).

Thus in the male tissues of these two kinds of flower-buds, the stage of development attained appeared to be the same. The female tissues of the abnormal bud were, however, much further advanced than those in the corresponding organs of the more normal flower of the same inflorescence.

In the material collected, 6th May, the two types of flower-buds were again found in the same inflorescence. _ The more normal flower-bud was similar /

similar in structure to that described above for flower-buds of 23rd. April collection; the stage of development of the male and female tissues has already been given (pp. 53, 57). Hence there would appear to be a greater discrepancy in the times taken for development by the male and female tissues of these buds than in the more normal buds of other inflorescences.

The large central flower-bud of this inflorescence showed marked differences from the lateral buds. Only four petals were present and these were free. The androecium consisted of seven stamens only but these were very large in comparison with those of the other buds of the inflorescence and bore very large anthers. Pollen-tetrads were present and the tapetum had collapsed. In many of the pollen-grains two nuclei were already showing while other tetrads showed more incompletely developed grains. (Fig. LI). The ovary was short and out of all proportion to the length of the flower and had grown up round the style to some extent so that the style seemed to penetrate into the centre of the ovary for some distance. The ovary was covered by a thick coating of hairs and possessed a very good vascular supply. Only four loculi were present, but each contained a number of fully formed ovules. (Fig. LVIII).

As with the male tissues the female tissues of /

of this species had taken less time for their development than is usual for members of the genus and consequently some abnormality was to be expected.

(VI) TABLE SHOWING CORRESPONDING STAGES IN DEVELOPMENT OF THE SEXUAL TISSUES:

The following Table shows the stages of development of the male and female tissues more clearly. In all cases, as already mentioned, the corresponding stages for the male and female tissues are taken from the same bud.

(VI) TABLE SHOWING CORRESPONDING STAGES OF DEVELOPMENT IN MALE AND FEMALE TISSUES.

	Date of Collection.	Male Tissues.	Female Tissues.
R. Keysii (material from normal flower-buds).	2/ 4/31.	Fully organised stamens, no sporogenous tissue.	Ovary formed but tissue undifferentiated.
	16/ 4/31.	Sporogenous tissue present; tapetal layer defined. Fig. XLIV.	Loculi defined; placental tissue indicated. (Fig. LII).
	17/ 4/31.	Larger area of active sporogenous tissue present; tapetum forming a single layer of cells in active division. (Fig. XLV).	Similar condition to that seen in 16/4/31. (Fig. LIII.).
	18/ 4/31.	Similar condition to that seen in 17/4/31.	Similar condition to that seen in 17/4/31.
	21/ 4/31.	Unequal development of pollen-sacs, some showing little tapetal and sporogenous tissues; others showing very large tapetal cells and disintegrating sporogenous tissue (Fig. XLVII.)	Loculi fully formed; placental tissue showing constrictions. (Fig.LIV).
	23/		

Table (continued).

	Date of Collection.	Male Tissues.	Female Tissues.
R. Keysii (Material from normal flower-buds).	23/4/31.	In some pollen-sacs fully-formed pollen-tetrads lying free with single-layered tapetum; in others disintegrating sporogenous tissue and large tapetal cells as in 21/4/31 (Fig. XLVIII).	Similar condition to that seen in 21/4/31.
	6/5/31.	Similar conditions to that seen in 21/4/31.	Similar condition to that seen in 17/4/31.
	14/5/31 (fresh material)	Pollen-tetrads well developed and very numerous.	Ovules well-developed and very numerous.
	27/5/31	Pollen-tetrads lying free in pollen-sacs; tapetum collapsed.	Placental constrictions more marked than in 21/4/31 - ovule rudiments larger (Fig.LV).
	4/6/31	Similar condition to that seen in 27/5/31.	Impossible to examine.
	10/6/31	Similar condition to that seen in 27/5/31.	Ovules definitely formed; embryo-sacs present. (Fig. LVI.).

Table (Continued)

	Date of Collection	Male Tissues.	Female Tissues.
R. Keysii (material from abnormal flower-buds)	23/4/31 (from same inflorescence as 23/4/31 above)	9 stamens present; pollen-tetrads fully formed; single-layered tapetum. (Fig. L.).	5 loculi; ovules fully formed; embryo sacs present. (Fig.LVII).
	6/5/31 (from same inflorescence as 6/5/31 above)	7 very large stamens present with very large anthers; pollen-tetrads fully formed; tapetum collapsed. (Fig.LI).	Ovary very short; 4 loculi; ovules fully formed. (Fig. LVIII).
	14/5/31 (from fresh material similar to 14/5/31 above)	Pollen-tetrads fully formed and lying free.	Ovules well developed.
	4/6/31 (same inflorescence as 4/6/31 above)	Impossible to examine.	Impossible to examine.

(VII) NOTE ON THE NATIVE HABITAT OF R. KEYSII.

It was thought that this delay in the initiation of the sexual tissues might be correlated with the environmental conditions of the native habitat of the species. The species is a native of Bhutan, found on the summits and northern ridges of the Hymalayas at an altitude of 8,000 to 10,000 feet, usually growing in spruce or mixed forest. The effect of the neighbouring trees was to prevent any direct sunlight reaching the plants, so that Keysii might be described as a "shade plant".

The winter in its native habitat is long and dry, the snow falling early in the season and lying until the spring. According to NUTTALL (30) who originally described the plant, "it grew amidst snows 2-3 feet in depth". One might thus conclude, in the absence of any evidence to the contrary, that, in its native environment, the cold had proved an inhibiting factor and hence all floral development had been compressed into one growing season, and that this rhythm of development was maintained on transference to more favourable conditions for growth, such as would obtain under glass.

However, on the continued examination of the plant during the autumn of 1931, it was found that flower-buds were present on Oct. 28th. These were very minute, the parts of the flower being recognisable /

recognisable only under the microscope, but there was no differentiation of any internal tissues. At the same date R. brachyanthum, grown under the same conditions, showed fully organised stamens and gynoecia with the male sporogenous tissue and the placental tissues well defined.

(VIII) DISTRIBUTION OF TANNIN IN THE FLOWER AND THE VASCULAR SUPPLY.

In the buds of the first collection made, 2nd. April, tannin was seen to be present in both the upper and lower epidermis of the petals, in some of the epidermal cells of the stamens and of the gynoecia and in isolated cells in the tissue of ovaries. (Fig. LIX.A.).

The amount of tannin present in the material of the next collection, 16th April, had greatly increased. It was present in both upper and lower epidermis of the petals, in the epidermal and sub-epidermal layers of the stamens, both in filaments and anthers, and as a sheath round the vascular bundle of the stamen; in the epidermal and sub-epidermal layers of the gynoecia and also in the cells forming the double walls of the loculi. The vascular tissue showed a regular arrangement in the ovarian tissue. (Fig. LIX.B.).

In the mature, open flower, the tannin, although /

although still occupying the same regions as in the immature flower, had, however, increased considerably in amount. In the stamen the tannin cells had formed a band, two to three cells thick, as a wall to the anther and also as a sheath around the vascular bundles. In the ovary the epidermal layers of tannin cells had formed a broad band, about four cells in thickness around the exterior of the ovary. Besides lining the loculi walls, the tannin layer had also extended around the periphery of the placentae and where the separation of the placentae extended into the centre, also lined the cavity so formed. Where the centre of the ovary consisted of a mass of tissue, the tannin was found in groups of cells forming a ring in the centre of the ovary. The vascular supply had increased in quantity and each bundle was ensheathed in a layer of tannin-containing cells. (Fig. LIX.C.).

(IX) SUMMARY:

In summing up the facts for a normal flowered inflorescence for the flowering period 1931, it was found that: -

(a) No winter dormant period occurred during floral development.

(b) The male and female tissues were initiated simultaneously in the spring immediately prior to the flowering period.

(c) The development of the floral organs was very rapid, two months only intervening between initiation and flowering.

(d) After the earliest stages the development of the male tissues proceeded more rapidly than that of the female, pollen-tetrads being fully formed before the ovules are delimited.

(e) As a consequence of (d) above, the male tissues had a brief dormant period before the bud was mature.

(f) The resulting pollen showed a large percentage of bad grains and the germination was poor.

(g) The growth of the floral organs had a slight retarding effect on the normal spring vegetative growth.

(h) The area of tannin-containing cells enlarged as development proceeded, finally ensheathing the vascular bundles completely.

(i) The vascular supply increased as development proceeded - in the stamens the single original bundle increased in size while, in the ovaries fresh bundles were initiated.

In the abnormal inflorescence the following facts were noted: -

(a) The central buds showed a variation from the normal ones in the size, form and number of the floral parts.

(b) The development of the sexual tissues in the central buds was more rapid and more complete than in other buds of the same inflorescence.

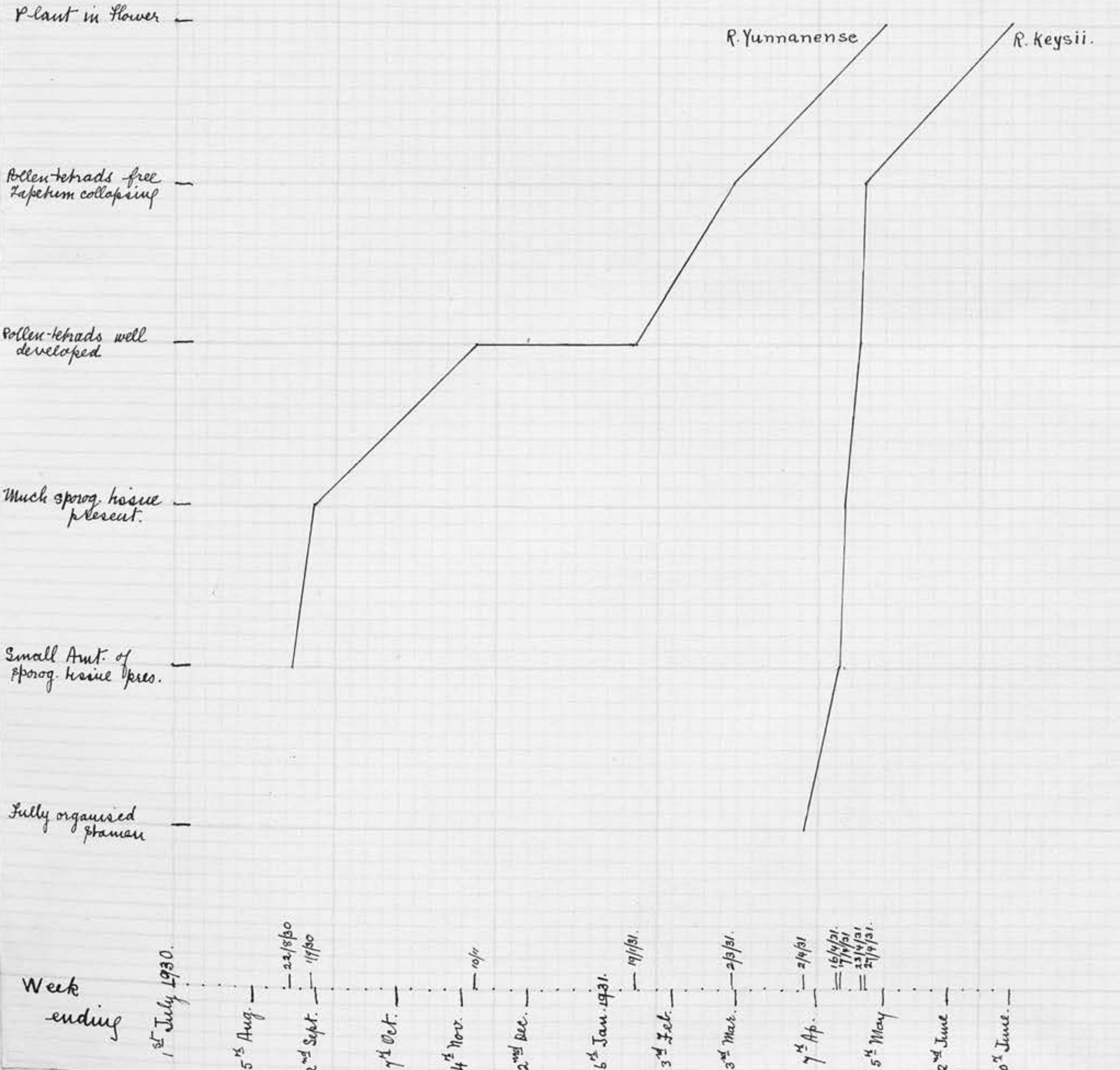
(c) The lateral more normal buds of these inflorescences showed a "lagging behind" of the female tissues, more than was evident in the buds of the normal inflorescences, - that is to say, the development of the female tissues was even slower than that of the corresponding tissues in the normal buds of an ordinary inflorescence.

(X) COMPARATIVE GRAPHS OF R. YUNNANENSE AND R. KEYSII:

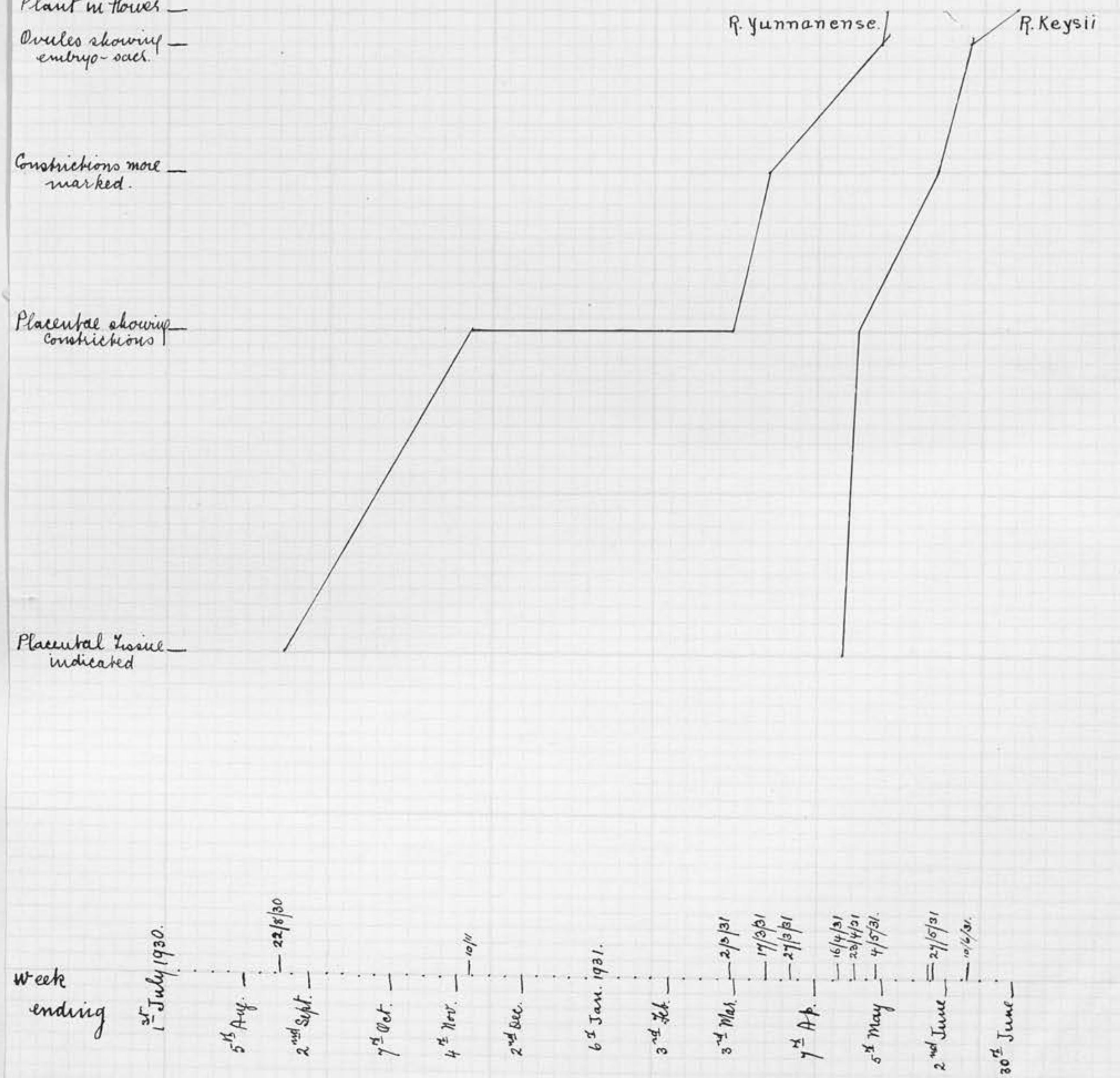
The deviation of the male and female cycles of development of R. Keysii from the normal cycle as typified in R. Yunnanense may be more clearly shown in the following graphs. The stages of development used are those which have been found to correspond during the course of development. The setting forth of these stages as requiring equal time for the development of each stage is purely arbitary, this point not having been sufficiently worked out to justify one in stating a definite time for the various developmental phases. However, sufficient data has been set forth to show the marked differences in the course of development of the male and female tissues of R. Keysii and R. Yunnanense.

Graphs over /

Development of ♂ Tissues of R. Yunnanense & R. Keysii.



Development of ♀ Tissues of R. Yunnanense & R. Keysii



VII. DISCUSSION.

- (i) Time and Floral Development.
  - (a) Length of Time required for Development of Sexual Tissues.
  - (b) Effect of different conditions on Time required for Development.
- (ii) Time and Sex Efficiency.
- (iii) Summary.

VII. DISCUSSION.

(i) TIME AND FLORAL DEVELOPMENT.

(a) Length of Time required for Development of Sexual Tissues:

From a study of the observations available on this point it is evident that the total time required for the development of these tissues, from their first initiation until their maturity, is very variable and is more or less bound up with the seasonable habit of the plant. From observations it has also been found that the rate of development varies, the male tissues attaining maturity in advance of the female tissues. One may roughly divide plants into two groups according to the time required for development, a grouping which is roughly correlated with the flowering period whether early or late in the season, but, of course, there are exceptions and variations in both groups.

Group I. The time required extends into two growing seasons, the plants being usually early, (i.e., spring) flowering.

Group II. The time required is within the limits of one growing season, the plants being usually summer-flowering or annuals.

Group I. With regard to Group I, the majority of plants included are most trees and shrubs, and the time required extends over a period of months, even up to eleven months; that is the initiation of the sexual /

sexual tissues for the flowering of one season is begun within one month of the flowering of the preceding year. Owing to the observed differences in the rate of development of the male and female tissues of any one plant, the full time of the developmental cycle is taken by the female tissues only in the case of protandrous flowers, the male tissues attaining maturity usually some considerable time before the completion of the cycle. Therefore, this cycle of development may be said to have two distinct phases, a predominantly male phase when the male tissues are developing more rapidly than the female, and a female phase, when the female tissues are still developing and the male, having attained maturity, are more or less in a dormant state.

If the male tissues of members of this group are considered alone, it is seen that their development proceeds steadily and uninterruptedly until the pollen-mother-cell stage is reached which is usually attained in the late autumn. COULTER and CHAMBERLAIN (16) in this connection write, "It would seem that, in general, those plants............develop their microsporangia before the end of the 'growing season' and that the mother-cell stage is the usual winter condition". (p. 31). Again these writers state (ibid), "The natural end of a growing season for the sporophyte would seem to be the attainment of the mother /

mother-cell stage by its sporangia which is really the limit of the sporophyte in the alternation of generations; and the natural beginning of the next season is the reduction division and the beginning of the gametophyte." (p. 31). In the above quotation the word "end" would appear to mean the 'cessation of' or 'slowing down of' activity vegetative and sporogenous. In other papers CHAMBERLAIN (10) states "most stamens pass the winter in the spore-mother-cell stage". (p. 171) and again, (11) "The spore-mother-cell seems to be a very usual halting place in the development of sporangia". (p.127).

B. M. DUGGAR (19) found that the pollen-mother-cells remained in a resting condition from October to early January, when activity was renewed and early February showed abundant divisions of the pollen-mother-cells.

A. H. CONRAD (14) notes the same fact with regard to <u>Quercus velutina</u>. In this species the male tissues pass the winter months as a "mass of apparently uniform cells", and that rapid development follows upon the "approach of a growing temperature" so that pollen is formed and shed about two months after the spring activity has begun.

From the data presented in these different papers to which reference has been made the following diagram was constructed which sets forth more clearly the /

the course of development of the male tissues.

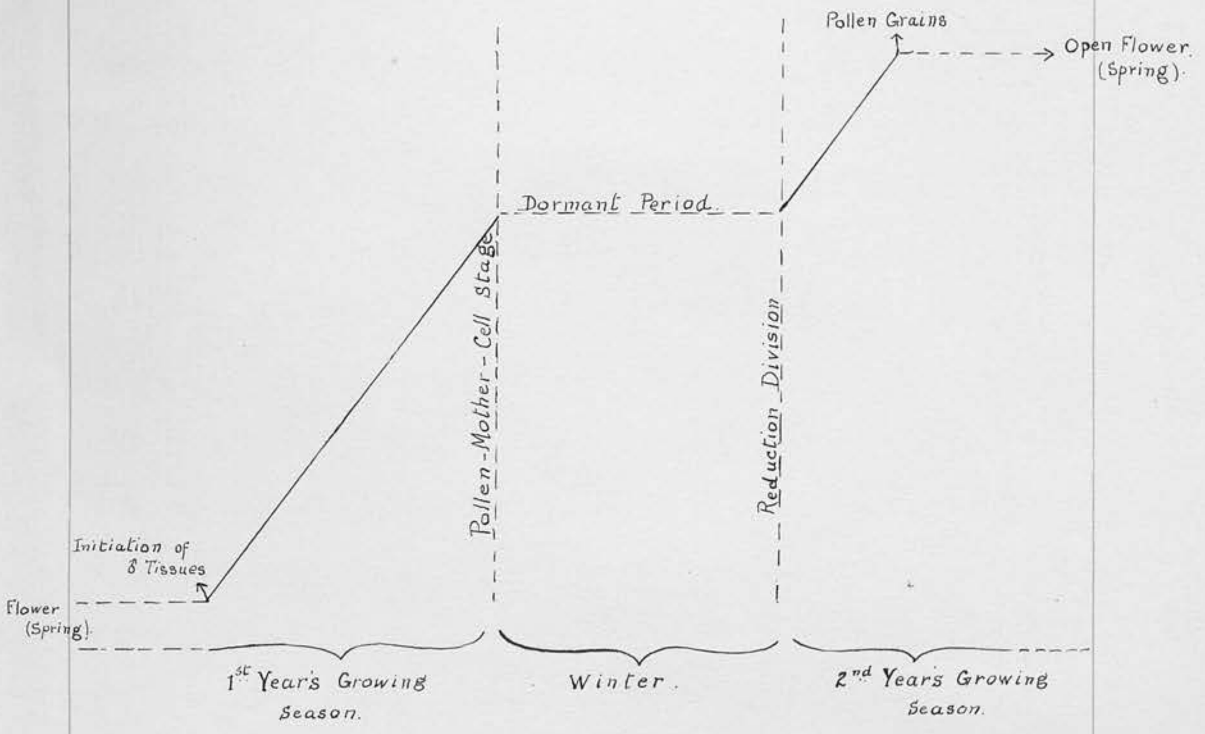


Diagram to show ♂ Development of Salix, Symplocarpus, Peltandra, Quercus - constructed from data given in above papers.

Although it would appear that the general rule for the male tissues is that the pollen-mother-cells form the winter dormant stage and the reduction division is the commencement of renewed growth in the spring, yet there are exceptions to this.

J. H. SCHAFFNER(36) observed that the bulbs of Erythronium began to develop the "incept of the flower" early in the summer and that by December 1st. pollen-tetrads were formed. The separation of the grains and the division of the nuclei of the grains might be delayed, but took place in the interval between December 1st. and April 1st. Thus the reduction division took place in the autumn and not after a winter dormant period. This may be express:ed diagrammatically as with the previous data, thus:

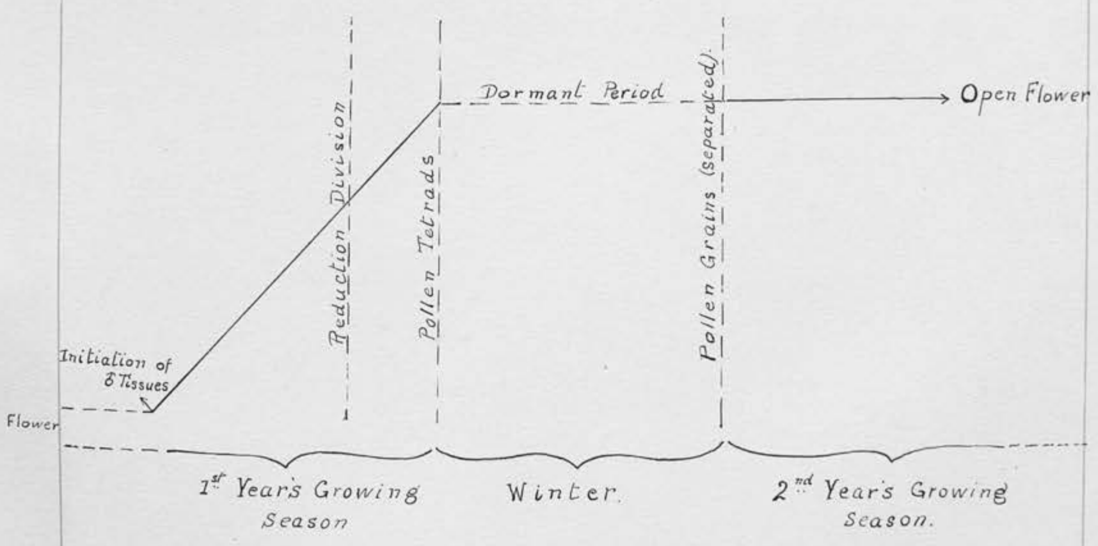


Diagram to show ♂ Development of Erythronium - constructed from data given in Schaffner's paper.

In the case of the members of the genus Rhododendron it was found that, with the exception of R. Keysii and possibly R. cinnabarinum, both of the Series Cinnabarinum, the winter condition of the male tissues of those species examined was the pollen-tetrad stage. The attainment of this stage was followed by a period of dormancy for the male tissues; in R. Yunnanense pollen-tetrads were found fully formed by October 21st. and a similar state was also found in the middle of January. In October the nuclei of the component grains of the tetrads were undivided; by March 2nd. the tapetal layer had partially collapsed and the individual nuclei of the grains had divided.

Expressed diagrammatically as before, the scheme of development of the male tissues for the majority of Rhododendron species is as given below:

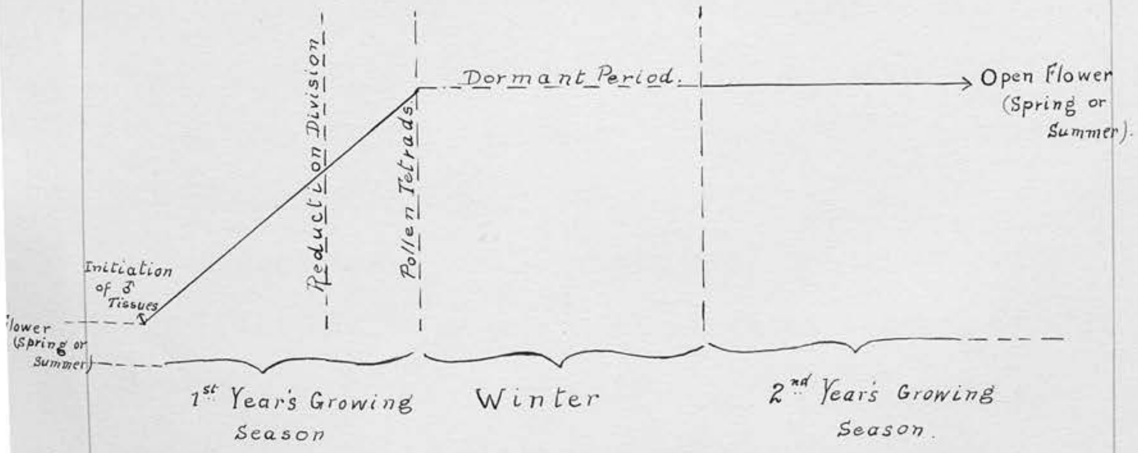


Diagram to show ♂ Development of Rhododendron spp. constructed from data presented in this paper.

The female tissues of the members of Group I., as has been already pointed out, are considerably slower than the male in their rate of development. COULTER and CHAMBERLAIN (16), speaking of the female tissues, say, "The length of time from the beginning of megasporangia to their maturity is very indefinitely known" (p. 52).

In Salix and Populus, CHAMBERLAIN (10) found that the megaspore-mother-cells did not appear until the renewed spring activity; the condition of the buds in October showed the nucellus defined but no integument present, the latter not appearing until spring. In Acer rubrum (29) the megaspore-mother-cell stage was found in March buds and this was presumably the winter condition. In Epigaea (16) the embryo-sacs were observed to be ready for fertilization in the autumn.

CONRAD (14) found that the female tissues of Quercus velutina passed the winter in the nucellar stage, the first indication of ovules being seen in the following spring, after a period of eleven months from the first initiation of the gynoecium.

SCHAFFNER (36) gives a more detailed account of the development of the female tissues in Erythronium. He observed the initiation of ovules by September, 1st. and the beginning of the integuments by October 1st., when the hypodermal archesporial cell could /

could also be distinguished. The reduction division took place in the early spring of the following year, the resting winter condition being the mega-spore-mother-cell.

As in the case of the male tissues, the data given above for the female tissues may be expressed in diagram form thus: -

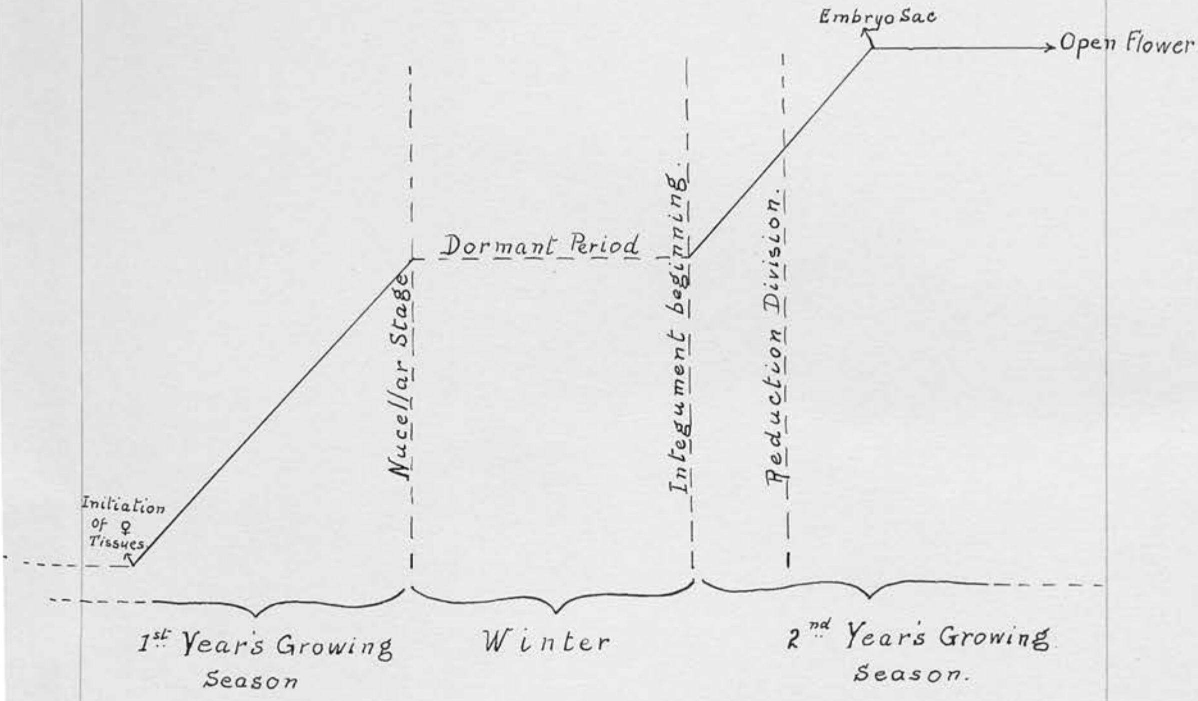


Diagram to show ♀ Development of Salix, Populus, and Quercus - constructed from data given in papers by Chamberlain and Conrad.

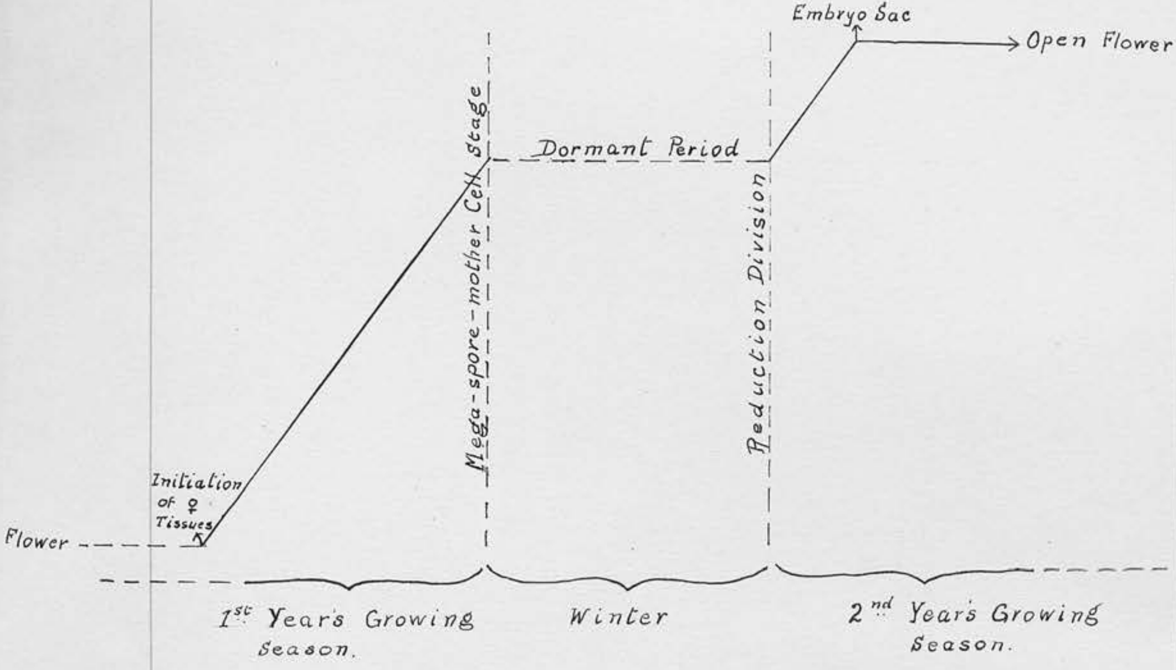


Diagram to show ♀ Development of Acer rubrum and Erythronium - constructed from data given in papers by Mottier and Schaffner.

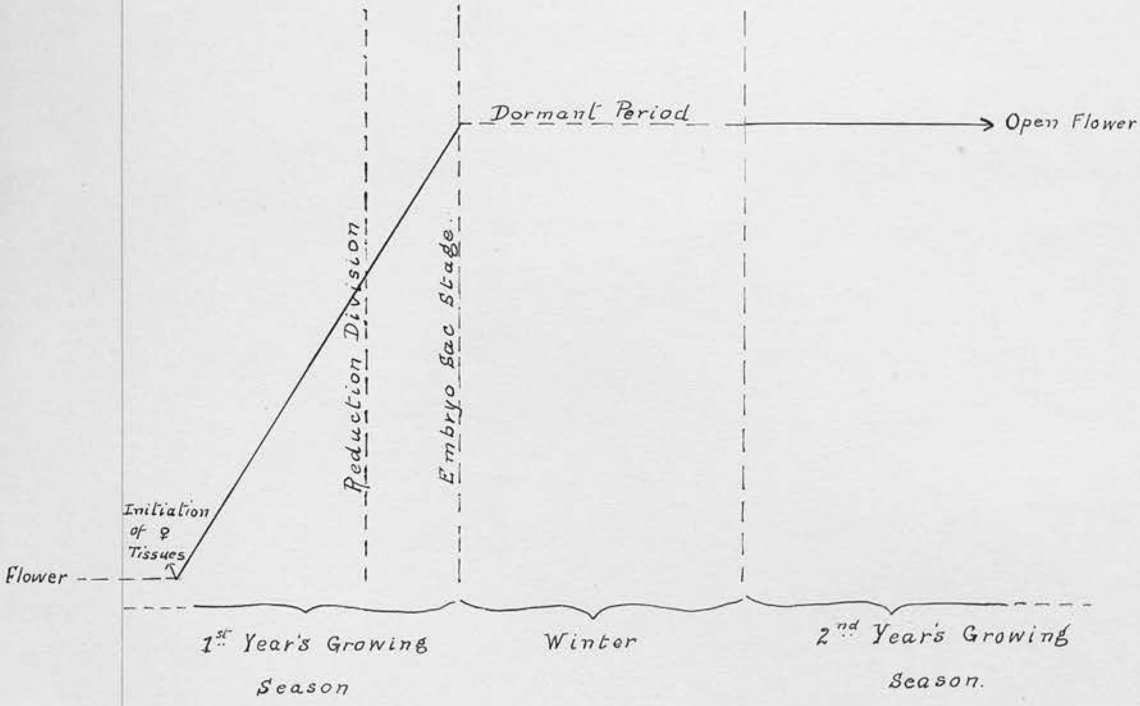


Diagram to show ♀ Development of Epigaea constructed from data given in paper by Chamberlain.

From the data presented in this paper the development of the female tissues of Rhododendron species would appear to be very similar to that of Salix, Populus and Quercus in regard to the time required. In the late autumn the condition in the ovary is that of well-defined placental tissue with ovular constrictions initiated. This is hardly as far /

far advanced as the "nucellar stage" found at this time in the above mentioned genera, but both stages agree in that there is no indication of any true female sporogenous tissue at this time. Some slight growth takes place during the winter period in Rhododendron, but the result is only an increase in area of tissues already formed; there is no evidence of any new developmental phase. From the recommencement of activity in the spring until the embryo-sac is fully formed there is steady and uninterrupted growth - that is over a period of about three months.

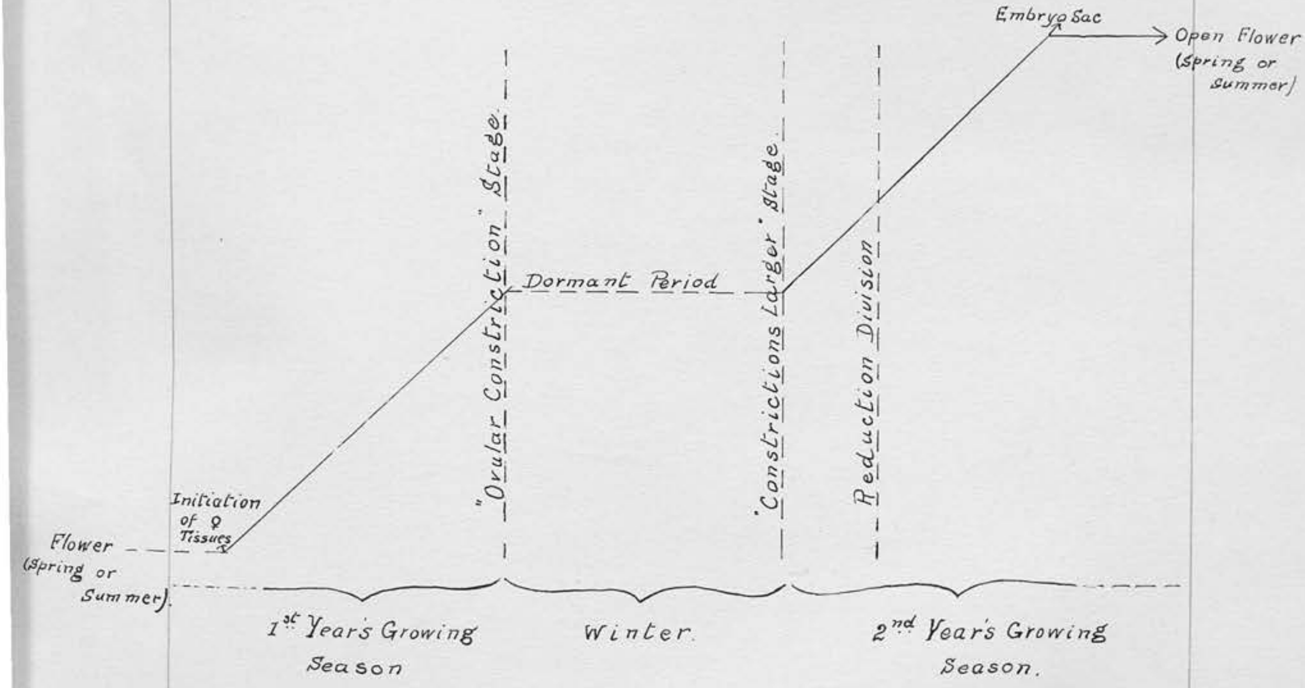


Diagram to show ♀ Development of Rhododendron spp. constructed from data presented in this paper.

Thus there would appear to be a considerable interval between the reduction divisions of the male and female-spore-mother-cells, with the exception of Epigaea. These reduction divisions mark, as it were, the ends of periods which are predominantly male and female though not exclusively so. In Salix, Populus, Quercus and Rhododendron the predominantly male phase is prior to the winter dormant period, the predominantly female phase commencing with the renewed spring activity. In Erythronium there is a tendency for the interval between the reduction divisions to be reduced and the male and female phases to be simultaneous. In Epigaea the interval between the reduction divisions, is further reduced and the male and female phases almost coincide and both are completed prior to the onset of the winter dormant period.

Group 2. In this group, where all the floral development takes place within the space of one growing season, the same distinction into a male and female phase, with slower development of the female tissues, may be observed. As the growing season does not extend over the winter months, there is no period of complete dormancy of the sexual tissues as a whole. Owing, however, to the difference in rates of development of the male and female tissues, each set of /

of tissues undergoes a period of more or less complete dormancy in turn.

R. O. WHYTE (43) makes a general statement, after the examination of several unrelated genera of flowering plants, to the effect that "........ a considerable interval always occurs between the reduction processes in the pollen-mother-cells and the megaspore-mother-cell in any given flower. Growth of the female tissues does not generally commence until complete tetrads are formed and pollen has been developed for some time before reduction in the ovules". (p. 189).

The term "female tissues" as used by WHYTE in the above quotation must be taken to refer to the ovular tissue only and not to the female organ as a whole with its contained specialised tissue which is the meaning given to the same term as used by the writer of the present paper and defined so in an earlier section.

The cycle of development of the male and female tissues as given by Whyte can be expressed diagrammatically thus: -

(Diagram over) /

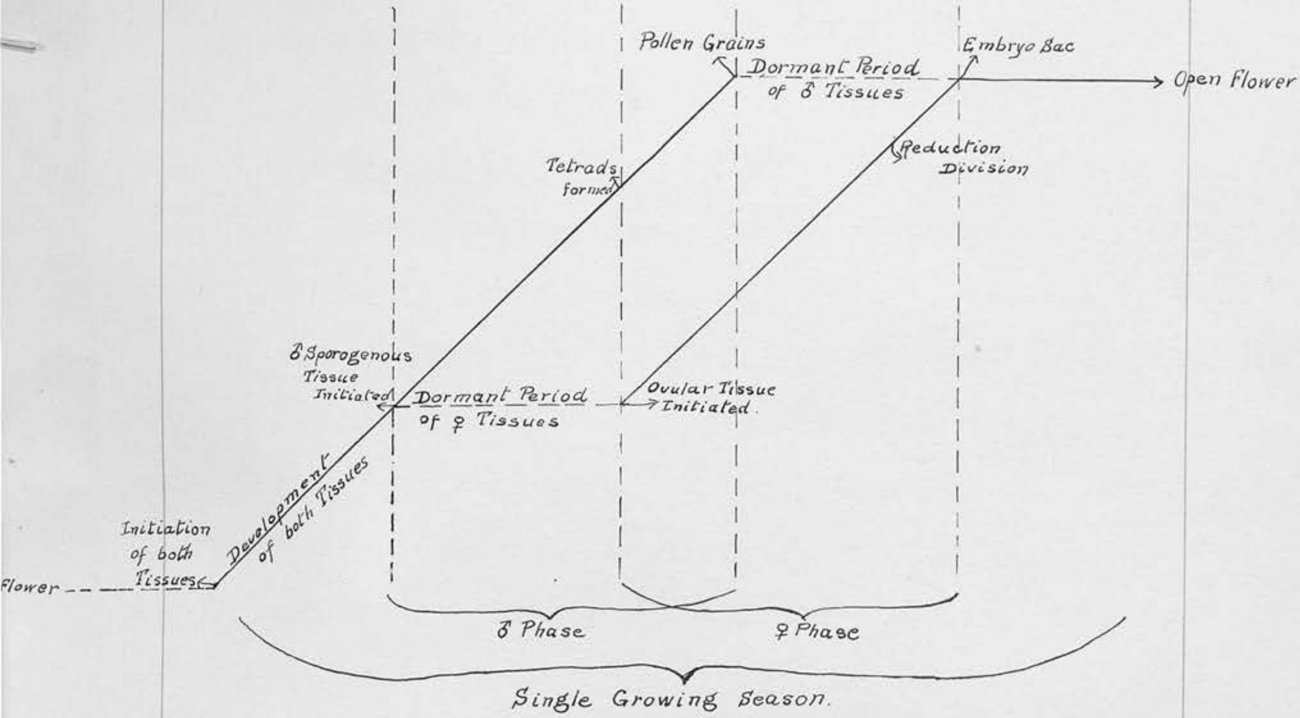


Diagram to show general Scheme of Development for ♂ and ♀ Tissues constructed from Whyte's data.

This general scheme of development WHYTE (42) points out "is the normal arrangement in hermaphrodite flowers, the interval between the two reduction divisions being constant for any given species". - (p. 413). Thus the boundary lines, as it were, of the male and female phases are the reduction divisions but a tendency for these phases to overlap is evident (see diagram). WHYTE found this to be the case in Silene maritima where the overlap was not great /

great and there was still a considerable interval between the reduction divisions.

In Ranunculus acris, WHYTE(43) found that in the normal hermaphrodite plant, the anthers had been completely finished and pollen formed before development commenced in the ovules of the same flower, the commencement of the male phase being considered to be when physiological activity began in the tapetum. The ovular development may commence slightly before pollen formation is complete, but usually it followed the last stages of pollen development. (See diagram below.).

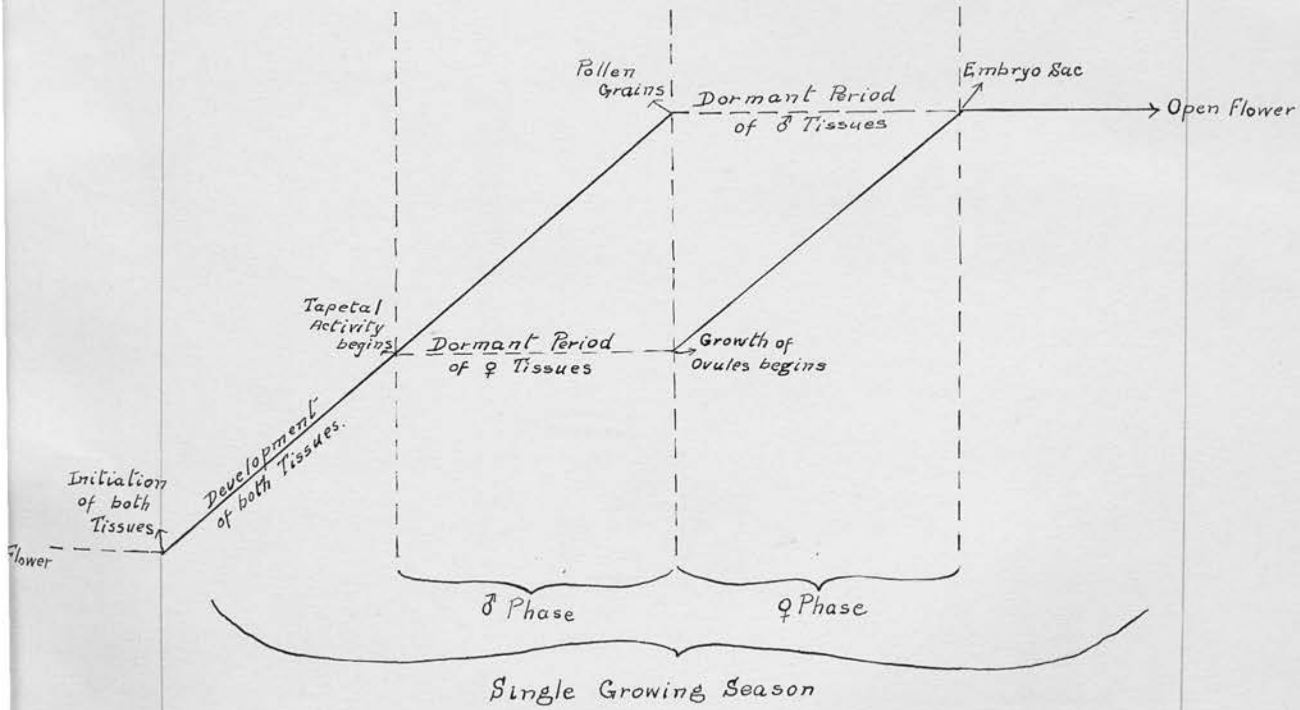


Diagram to show ♂ and ♀ Development of Ranunculus, constructed from data in Whyte's paper.

Therefore in Ranunculus there is "an interval of considerable duration between reduction in the male and female organs of the same flower". (p. 185). Thus the tendency to an overlap of the two phases is practically eliminated.

During the course of this study on members of the genus Rhododendron one species, R.Keysii, was found where the development of the male and female tissues took place within the one growing season. Full details of this development are given in a previous section of the paper. It may be noted, however, that the usual interval between the reduction divisions in this genus, an interval of eight to ten months, is here reduced to some weeks, the whole period of development, from initiation of the organs to full maturity of the tissues, only occupying some two months instead of eight to ten months as in other species of the genus. The female tissues do not undergo any period of dormancy during the whole cycle of development, while the development of the male tissues particularly and the initiation of the organs as a whole has been hindered. This point is brought out more clearly in the accompanying diagram.

(Diagram over) /

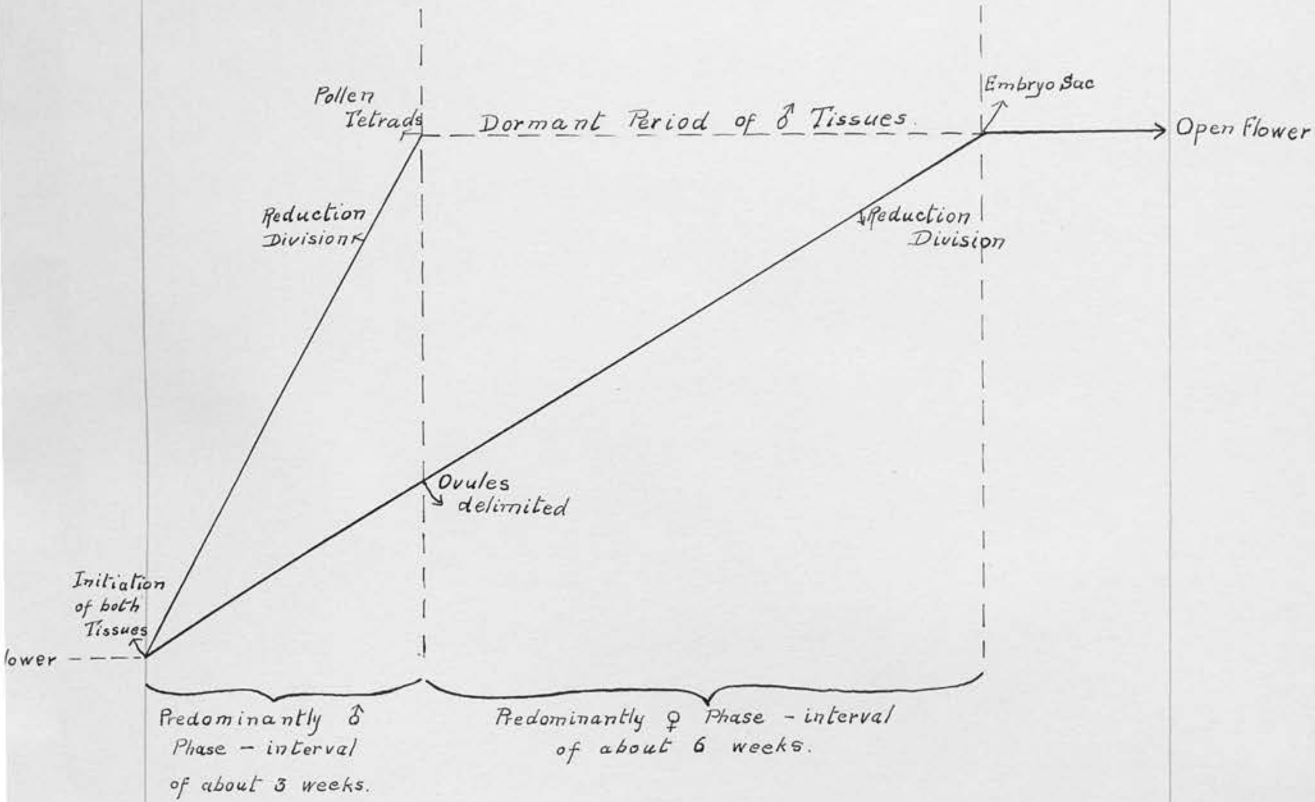


Diagram to show ♂ and ♀ Development of <u>R. Keysii</u> constructed from data given in this paper.

In <u>Centaurea</u> (central disk florets only) and <u>Triticum</u>, WHYTE (47) found that the interval between the reduction divisions was even less, these divisions proceeding concurrently in normal hermaphrodite flowers/

flowers; while he found the reduction divisions coinciding in *Plantago* and *Veronica*. These last two genera are protogynous, so that the time required for the female tissues to attain maturity after the reduction division had taken place would be less than that required by the male tissues. Whether this only happens in protogynous flowers or is always the case in any flower, or whether, even in protogynous flowers, the complete cycle of development of the female tissues is longer than that for the male has not been investigated as yet. Presumably the female organs of a protogynous flower are initiated simultaneously with the male ones as in protandrous flowers, so that it is fairly probable that the complete female cycle has been shortened.

(b) Effect of different Conditions on Time required for Development:

1. Growth of other parts of the Plant.

It has already been noted that the seasonal habit of the plant has a distinct relation to the time required for the development of the sexual tissues. But, as has already been shown in this paper, in the case of the genus *Rhododendron* the vegeta~~bl~~tive growth of the plants has no effect on the floral development. Rather is the reverse the case, and the floral development proceeding simultaneously with /

with the vegative growth hinders the latter, as for example in the flowering and non-flowering plants of R. Keysii. Under natural conditions the vegetative growth and the floral development in many Rhododendrons proceed simultaneously. Even where the vegetative growth appeared to be finished prior to flowering as in the summer-flowering species, the stages attained in the new floral development prior to the onset of the winter dormant period in the majority of cases were the same as those attained by the spring-flowering species. So that the time interval between the reduction divisions in the male and female tissues in any species is not affected by the vegetative growth. WHYTE (43) referring to Ranunculus acris writes that the interval between the reduction processes is probably governed largely by the amount of ovular development in the plant concerned; where there is a small amount of ovular development, as in Centaurea, the reduction processes tend to coincide. This ovular development or "the very considerable somatic growth associated with ovule development in Ranunculus" (p. 108) as he calls it in a later paper (47) but presumably meaning the same thing, must therefore proceed simultaneously with pollen development. But he found flowers of Ranunculus differing from the normal in that the pollen was not developed, in which the reduction /

reduction processes were found to coincide. He does not mention the "somatic growth" in connection with these flowers, so that one may presume it was normal as were the female tissues themselves. Hence since the ovular development altogether was normal and yet the reduction divisions coincided it cannot be the amount of ovular development which governs the interval between the reduction divisions as he stated previously.

Again, if the amount of ovular development governed the time interval between reduction divisions, then in the case of R. Keysii, where the interval was reduced by many months, the amount of ovular development should have been very small, but it was found to be the contrary and the number of ovules formed appeared to be the normal.

2. Food Supply.

WHYTE(43) again writes, "The organisation of the flower cannot adapt itself to the physiological strain entailed by the simultaneous development of pollen-mother-cells in the anther and archesporial cells in the ovules, and that, as a result, abortion of one or other of the reproductive parts of the flower follows". (p 190). This statement is further elaborated by an examination of other unrelated genera, in all of which /

which any abortion or abnormality of floral parts is explained by a lack of a sufficient food supply. In Saxifraga potternensis and Silene maritima he refers the abortion of male tissues to the inadequate food supply (in the latter case brought about by a "dry" season) but does not mention the time interval in either case.

This would indicate that the reason for a successive development of the reproductive organs and a consequent slowness of development of the female tissues was a physiological one - the question of an adequate food supply - and that the time interval between the male and female reduction divisions was shorter or longer according as the food supply was abundant or scarce. If then the food supply were abundant there would be manifest a tendency for the reduction divisions to approach each other in time and, under optimum conditions of food supply (other conditions being favourable), the reduction divisions would coincide, and the development of the tissues be simultaneous.

In the species of Rhododendron studied, those plants grown under glass, may be assumed to be under conditions approaching the optimum for growth. However, when plants of the same species, grown under glass and in the open, were compared, e.g., R. Yunnanense/

R. Yunnanense, R. arboreum, R. calophytum and others, it was found that the amount of "speeding up" of the growth, or, in other words, shortening of the time interval, shown in the glass-house plant was very little and hardly to be taken into account when the length of time from the first initiation of the male and female tissues to their full maturity in the opened flower was considered. Also the dormant period was still present in the floral cycle. These glass-house plants still apparently required from eight to ten months for full floral development.

In only the one species, R. Keysii, as already detailed in another section, was there any alteration in the time-interval. The alteration here, as has already been shown was not a case of "speeding up" of development and thus a shortening of the time interval from the female end as it were, but rather a retarding of development particularly of the male tissues, the whole cycle of floral development being compressed into a few months prior to flowering, instead of being spread over the eight to ten months before flowering as in other species of the genus. Also this delay in development took place when the plant was being grown under presumably optimum conditions. It was noticeable also that the vascular supply increased as development proceeded, the /

the original vascular bundle in the male organ increasing in size while in the female organ fresh bundles were initiated. But, whereas there was thus an increase in vascular supply in <u>both</u> tissues and this increase took place after the shortening of the time-interval, or, more correctly after the retarding of the male development, it cannot be said to be the cause of the disturbance in the time interval.

GREGORY (21) found that there was no obvious difference between the development of the vascular strands in the fertile and sterile stamens.

Therefore the delay in development must have been due to some cause other than the vascular supply.

WHYTE (42) also states, referring to *Ranunculus*, that the interval between the two reduction divisions is constant for any given species. As the author does not mention the inadequacy or otherwise of the food supply in this connection, one assumes, therefore, that in *Ranunculus* the time interval is a constant for any species irrespective of the food supply, contrary to other statements made by this author.

3. Size and Number of Flowers produced.

The time required for development of the male and female tissues does not appear to be affected by /

by the size and number of the flowers produced.

The flowers of R. calophytum, a plant grown under glass are very large: corolla 5-6 c.ms long, 5-7 lobed with posterior lobe 2-2.5 c.ms long and 3-3.5 c.ms broad; stamens 15-20, 1.5-3 c.ms. long; pistil about 4.5 c.ms long and ovary 6 m.m. long. These flowers occur in many-flowered racemes, up to thirty flowers. The flowers of R. brachyanthum, another species grown under glass, are much smaller: corolla about 1-1.5 c.ms long, 5 lobed; stamens 10; pistil short and ovary about 2 m.ms. long. The inflorescence is a raceme containing three to four flowers. The former plant requires about eight months and the latter about 10 months for the full development of their flowers under glass-house conditions. In the open the flowers of both species were about three weeks later in opening.

The flowers of R. Keysii are, roughly, about the same size as those of R. brachyanthum, but more t~~a~~ubular. The former, as has already been noted, take a few months only to develop, and not only is this the case for the normal flowers, but also for the large, abnormal flower found in several of the inflorescences.

4. Temperature.

The temperature, on the other hand, would appear under /

under certain conditions, to have an effect on the development of the tissues. Under glass-house conditions the temperature varies as the outside temperature but does not fall quite so low so that danger of frost is precluded. The different species of Rhododendron, it was observed, flowered a little earlier when grown under glass than when growing outside. This was to be expected since the majority of species occupy a more or less mountainous habitat when growing wild and therefore would be subject to considerable changes in temperature, so that when introduced into a habitat where the temperature is, on the whole, higher than in their native districts the flowering is a little quicker, the interval between the reduction divisions is therefore lessened causing the "speeding up" of the female development, but having little or no effect on the male tissues.

Unseasonable temperature changes, however, particularly when these are tending to be higher temperatures, have a more marked effect and influence the male as well as the female tissues. In the late autumn of 1931, in the middle of October, there occurred a period of mild weather, the temperature being higher than for some time previously. On Oct. 21st. a large number of species both in the glass-house and outside was examined and all were found to contain pollen-tetrads fully formed and lying /

lying free, with the exception of R. cinnabarinum. In this species growing outside at this date, pollen-mother-cells were found.

During the following week a fall in temperature was experienced. Another examination of R.cinnabarinum was made on October 28th, when it was observed that within the same inflorescence-bud, there were flower-buds containing pollen-mother-cells and others containing pollen-tetrads, these last lying in a mass with a certain amount of undifferentiated tissue. It would thus appear that the mild temperature had favoured continued development and had induced the development of pollen-mother-cells to pollen-tetrads in this particular species. The fact that this change in the male tissues in cinnabarinum took place towards the end of the month, much later than in other species growing outside, and towards the end of the mild weather period, and was incomplete even then, would indicate that the pollen-mother-cells were the winter condition of the male tissues of this species and the mild temperature had had a forcing effect. At the same time, Oct. 28th., it was noted that minute flower-buds, absent on October 21st. were beginning to develop on R.Keysii in the glass-house contrary to what had been found the previous season.

R. cinnabarinum/

R. cinnabarinum has a similar distribution to that of *R. Keysii*, but attains to higher altitudes, 10,000 to 12,500 feet. The former species would, therefore, endure a lower temperature than *Keysii*, and milder temperatures would have a correspondingly greater effect. Added to this is the fact that *R. Keysii* and *R. cinnabarinum* are the sole members of the Series Cinnabarinum,(34) "a small and very distinct group confined to the Eastern Himalaya and without its counterpart in China or elsewhere...... The true affinity of the group is not very obvious and it stands somewhat isolated in the lepidote-leaved section". (p. 221).

Thus in both these species there had been a "speeding up" of the male tissues owing to the unseasonable higher temperature.

It has also been noted by gardeners and others that unseasonable high temperatures hasten the development of the Rhododendron flowers so that the spring blooming may be antedated and take place in the winter or late autumn preceding. That is to say the time-interval between the reduction divisions has been lessened and the development of the female tissues has been hastened by the more favourable temperature.

Unseasonable low temperatures, particularly if there /

there is a sudden drop from a fairly high temperature, result oftentimes in frosting of the buds; but, when frosting does not occur, the low temperature appears to have little effect on the development. The early spring of 1931 was extremely cold, snow fell on Feb. 28th and continued off and on for ten days, lying thick on the ground the whole time. It was noted that R. Yunnanense, growing outside, was in partial flower (a number of flowers were open, but no inflorescence was completely out) by May 4th of that year. For the previous two years, 1929, 1930, it was recorded as flowering outside during May. The same species grown under glass, had flowering records for these years - 1929, flowering first week in May; 1930, flowering during May. Hence the unseasonable low temperature of early spring had had no effect on the flowering of the plants in the open. The same point was noted with regard to other species grown outside.

The temperature, then, has little or no effect on the development of the male and female tissues, unless high temperatures occur unseasonably when development is hastened.

(ii) TIME AND SEX EFFICIENCY:

R. O. WHYTE in several papers on Ranunculus,(43) Silene(44) and particularly in that on Saxifraga potternensis,(47) goes into the matter of the time interval between the reduction divisions in some detail. He points out that where the normal time interval for a plant has been shortened one or other of the reproductive parts of the flower shows ab~~s~~ortion. The commonest abortion found during the course of his investigations was what he termed "anther deficiency", the term "deficiency" being defined by him as covering "true reduction of floral parts, either in size or stage of development reached". (47, - p. 107). In Saxifraga potternensis(47) (F_1 generation) he found "anther deficient" flowers which showed -

(a) normal sporogenous tissue and tapetum, - in a few cases pollen being formed;

or

(b) normal pollen-mother-cells and tapetum, - but inability to develop further;

or

(c) normal pollen-mother-cells and inactive tapetum;

or

(d) disorganised sporogenous tissue and disintegrating tapetum, while the ovule development in the same flowers was found to be quite normal.

The /

The reason he puts forward for the existence of these "anther deficient" flowers and the few normal ones is that there is some influence in the plant which causes anther degeneration. "The great preponderance of anther-deficient flowers suggests that the occurrence of a few flowers (not more than two or three were noted) with good pollen is to be regarded as an exception due to the temporary absence of the influence which causes this anther degeneration". (loc. cit. p. 102). In the same paragraph he states that the pollen-mother-cells fail to develop "owing to the lack of suitable supplies from the tapetum at the critical juncture". (loc. cit.).

Referring to flowers of this same species which showed reduced ovaries as well as "anther deficiency" he cannot give any reason for these abnormalities but suggests that either competitive development of the floral parts or a choked vascular supply may be the cause.

In connection with this plant, Saxifraga potternensis, he does not mention the time interval between the reduction divisions at all, in spite of his assertion in a previous paper (44) - "The influence of a time factor has already been shown to play a large part in the various sex forms of Ranunculus acris (Whyte, 1929).......... In certain plants, the respective /

respective periods of activity have come to overlap to a greater or less extent, with a failure in the anthers in proportion to the amount of this overlap, thus giving rise to all intermediate forms between a normal hermaphrodite and a 'female' flower. This influence is present also in Silene maritima (Plant A_2) causing male sterility". (p. 198). And again, in the same paper, ... "the time factor had become operative and reduced a low pollen output to nil by reduction of the period of activity in the tapetum". (-p.199).

(47)

Also in his paper on Saxifraga potternensis, referring to his previous work on Silene and Ranunculus, WHYTE writes, ..."a 'time factor' was postulated to account for certain abberant types of floral morphology. The hypothesis of competitive development was found to explain the phenomena tolerably well, and has since been applied to similar abnormalities in other genera". (p. 107). In this paper, too, he deals with other plants in detail and gives a variety of reasons for the sexual abnormal-:ities observed. These may be briefly enumerated: -

Silene maritima possessed flowers showing "mild anther deficiency" which became "totally anther-deficient without pollen" on experiencing a "dry" summer season.

Aesculus/

Aesculus showed ovule deficiency, the reduction divisions proceeding concurrently.

Asparagus showed all types of "deficiency", both anther and ovule deficiency and intermediate stages, the reduction processes coinciding and thus, according to the writer, providing the necessary condition for the occurrence of deficiency.

Bomarea, "Anther deficiency" in one stamen due to its developing phase having overlapped into the ovule developing phase.

The types of deficiency in the above-mentioned flowers as also in Ranunculus and Saxifraga is, generally speaking, held by WHYTE to be due to "fundamental defects in the organisation of the plants or the parts of the plants concerned" (loc. cit. p. 114).

Other plants mentioned in this paper of which details are given are: -

Nolana showing "anther-deficiency", the anther development being governed by the condition of the tapetum. This is not considered by the author to be "true anther-deficiency" but to be due to temporary local conditions such as wilting, lack of sufficient light and so on. "True anther deficiency" is here defined by the author as being due "to a fundamental inability to achieve the reduction division" (p.114).

Fragaria/

Fragaria showing "anther deficiency".

Rubus idaeus showing ovule deficiency.

Rumex alpinus precise abnormality not given.

Empetrum nigrum " " " "

The main cause assigned by WHYTE for the production of the abnormalities evident in the last four species which are referred to as "true floral deficiency" is competitive development.

To sum up Whyte's points on "deficiency" as set forth in his various papers: -

There are two kinds of "deficiency" which he distinguishes: -

(a) "Deficiency" due to fundamental ability to achieve the reduction division - e.g., *Ranunculus*, *Saxifraga*, *Silene*, *Aesculus*, *Bomarea*, *Fragaria*, *Rubus*, *Rumex*, *Empetrum*.

(b) "Deficiency" due to temporary local conditions such as wilting, etc., - e.g., *Nolana*.

In (a) the "deficiency" is caused by: -

(i) Alteration of time interval between reduction divisions having a direct effect on the tapetum, e.g., *Ranunculus*, *Silene*, *Aesculus*, *Asparagus*, *Bomarea*.

(ii) Competitive development arising from inequalities in the food supply, e.g., *Ranunculus*, *Silene*, *Saxifraga*, *Aesculus*, *Fragaria*, *Rubus*, *Rumex*, *Empetrum*.

(iii) /

(iii) chocked vascular supply, e.g., Saxifraga.

(iv) "dry" summer season, e.g., Silene.

In (b) the author asserts that the temporary local condition produces "deficiency" through the deleterious effect the unfavourable condition has on the tapetum.

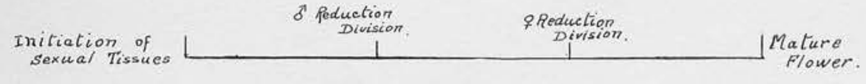
One would naturally think that the "anther deficiency" produced in Silene by a "dry" season would belong to the second class of deficiency as given above, but the author definitely states (p.114) that the deficiency in Silene is due to fundamental defects in the organisation of the plant. If the "deficiency" is fundamental then no matter how the local conditions are altered this "fundamental" type of deficiency cannot be altered and is therefore independent of external conditions.

Again in connection with the alteration of the time interval between reduction divisions, Whyte writes "this change has a retarding effect upon the anthers, which is expressed in widespread tapetal failure ...... etc." (p. 108) Would it not be more logical to say the anthers are retarded in development and therefore a change of the time interval results?

It /

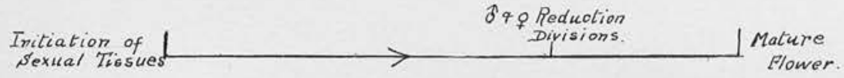
It is also quite conceivable that the alteration in the time interval may be due to a "speeding up" of the female development phase. To express the extremes of these ideas more clearly, they may be set forth diagrammatically thus: -

A.



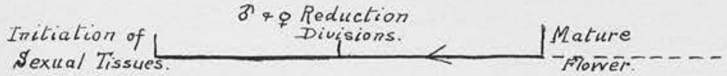
Normal ☿ Protandrous Flower.

B.



Retarding of ♂ Development.

C.



Hastening of ♀ Developmen

Of the above diagrams, A. represents the conditions of development in a normal hermaphrodite protandrous flower. B. and C. are the conditions of development in a hermaphrodite protandrous flower when there occurs a change in the time-interval between the reduction divisions, the reduction divisions tending to coincide. The coincidence of the reduction divisions and the resulting /

resulting change in the time-interval has, however, been brought about in two different ways in B. and C. In B. there has been a retarding of the development of the male tissues and so a shortening of the time-interval from the male end as it were; while in C. there has been a hastening or "speeding up" of the development of the female tissues so that the interval is shortened from the female end and the flower opens prematurely.

In the course of this study on the genus Rhododendron the condition obtaining in A. was seen in the majority of the species, typically in R. Yunnanense; a condition approaching that seen in B. was observed in R. Keysii; while a tendency towards the third condition is seen when the plants flower prematurely as happens when they are subjected to unseasonable high temperatures.

One gathers from Whyte's papers, that the interaction of the time-interval change and the tapetum is the controlling factor in sexual abnormalities; but one also finds that in these papers, the tapetum may be called upon to influence the sexual tissues from various angles: -

(a) through the shortening of the period of its activity owing to time-interval change and, as a result, a "low pollen output is reduced to nil", e.g., Silene.

(b) through its inability to provide suitable supplies /

supplies when most needed by the male tissues (no reason being given why this should be so), e.g., Saxifraga.

(c) through its more or less complete failure owing to temporary local conditions at any time during pollen development and hence the pollen output is reduced accordingly, e.g., Nolana.

(d) through its failure owing to the change in the time - interval between the reduction divisions, e.g., Ranunculus.

The consequent effect of this tapetal influence, however it may be applied, is a lessening of the pollen output and this, the author asserts is inversly proportional to the amount of female tissue developed owing to the balance of the development of male and female tissues having been upset and competition having thus entered into the matter.

But in certain cases, e.g., Saxifraga, flowers were found showing "ovule deficiency". This the author correlates with "petal deficiency"; but, sometimes, "anther deficiency" was found together with "ovule deficiency" and no "petal deficiency". No mention of the tapetum or the time-interval is made in this connection, the reason for the abnormalities being given as inequalities in the vascular supply to the various parts concerned.

WHYTE concludes his paper on Saxifraga potternensis (47) with the statement that the "origin of the 'deficient' forms of floral morphology" is competitive /

competitive development, the factor introducing "this competitive influence is conceivably a reduced nutrition level in flowers; a subsidiary factor is the high metabolism rate necessary for meiosis" (p. 120).

This statement is thus a contradiction of the statements made in earlier papers to the effect that: "It is conceivable that this time factor will explain the occurrence of complete and partial dioecism in many species". (p. 413).(42)

"The 'time factor', which was found to explain the occurrence of various sex-forms of *Ranunculus acris* (Whyte, 1929) applies also to a similar question in *Silene maritima*" (p. 200).(44)

"The incipient sterility noted in the hermaphrodite plants of this species and of *S. vulgaris* is due to the effect of the time factor in a milder form". (ibid. p. 200).(44)

"The position of *S. vulgaris* on the table indicates that incipient sterility is still in an early stage, that the time interval between male and female reduction processes has deviated only very slightly from that of a normal hermaphrodite *silene*". (ibid. p. 199).(44).

"The amount of pollen produced is entirely dependent upon the duration of activity in the tapetum /

tapetum, and this in turn has been shown to be directly dependent upon the length of the interval between male and female reduction processes. If there is no interval, there is no activity in the tapetum. If there is an interval intermediate between this state and the normal for a hermaphrodite flower, tapetal activity will go on until checked by the growth of the ovules, and a corresponding pollen development will be found". (p. 190.)(43).

In the course of this study it has been shown that the cycle of development of the sexual tissues for the majority of the species of Rhododendron is exceedingly uniform throughout the genus and extends over two growing periods; and that a definite interval of time, varying according to the species, elapses between the flowering period of one year and the attainment of any stage in the development of the floral organs for the succeeding year. At the onset of the winter dormant period, however, the pollen-tetrad stage and the placental stage showing ovular constrictions are the conditions of the respective sexual tissues for the majority of the species and, therefore, there is a certain definite time-interval between the reduction divisions.

If then, there is any alteration in the time-interval between the reduction divisions caused either /

either by a hastening or retarding of development, the sexual tissues will tend to attain maturity at an unseasonable time; and since maturity tends to be attained unnaturally, the tendency is for some abnormality to be shown in the resulting flower.

In R. Keysii (collections prior to flowering 1931) such an alteration in the time-interval was evident and the abnormality noticed was a tendency to sterility on the part of the male tissues, only about 19% of good pollen being present. In this case there had been a retarding of the development of the male tissues and a consequent lessening of the time-interval between the reduction divisions (See Diag. B. p. 107). A certain amount of seed was set as the result of this flowering season; but, on being placed in a germinating oven for several weeks it failed to germinate showing that the gametes were both more or less inefficient in their action.

The result for 1932 from this species cannot yet be given.

GREGORY (21) found that there was a delay in development in the pollen-mother-cells in the sterile plants of Lathyrus odoratus, compared with the condition found in the fertile plants. In the latter the active divisions of the archesporial cells were followed by a period of rest and growth. In the sterile /

sterile plants the rapid growth of the archesporium was, however, succeeded by an abnormally long period during which no divisions of the pollen-mother-cells took place. The structure of all the embryo sac mother-cells was quite regular. The resulting sterility was confined to the male organs. In other words the time-interval between the reduction divisions had been shortened from the male end by a retarding of the development of the male tissues and no pollen grains were formed.

DAVEY and GIBSON (18) in a note on the sexuality of Myrica Gale write - "Since the staminate flowers are developed early in the season preceding that in which they flower, while the pistillate catkins develop much later, it is possible that conditions accelerating or retarding the development of catkin buds may influence the proportions of the two kinds". (pp. 150-151).

It may be assumed here that the regular recurrence of the reduction divisions in the development of the sexual tissues in any plant is due to some inherent factor in the plant itself. If the plant is healthy and the conditions of its environment are normal and suitable, the various phases of the plant's life processes proceed normally and the reduction divisions take place in regular sequence /

sequence; in a hermaphrodite, protandrous flower the male tissues are affected first followed, after a longer or shorter interval, by the effect on the female tissues. If this inherent factor is present in a sufficient strength and of a suitable quality, as it would be in a normal healthy plant, then the time-interval between the reduction divisions is constant for that particular plant and so is the time - interval elapsing between the flowering of one season and that of the next season.

If, however, the plant itself were normal but the conditions became abnormal, then this inherent factor would tend to exert its influence at the certain definite time as usual, but the abnormal conditions would have tended to upset the life-processes of the plant so that there would be either a hastening or a retarding of sexual development. The result would be -

(a) either the inherent factor would not be present at the altered time in sufficient strength and quality,

(b) /

(b) or the sexual tissues on which the inherent factor has an influence, would not be in a suitable state at the altered time to react normally to this influence.

(c) or neither the inherent factor nor the sexual tissues would be in a suitable state at the altered time to proceed normally.

The consequence of any of these alternatives following the hastening or retarding of development of the sexual tissues with a change in the time-interval between the reduction divisions as a natural outcome of this alteration in development, would be some abnormality in the sexual tissues, the tendency being towards sterility, since either the inherent factor or the sexual tissues are deficient in some way.

WHYTE, as has already been pointed out, brings forward a number of circumstances - time-factor, food supply, external local conditions, competitive development, etc. - any one of which may apparently account for this sterility or "deficiency". LATTER (26) favours Gregory's view that the sterility of the male tissues is an expression of "some deep-lying phenomenon which affects the physiology of the plant". (p. 295).

This statement is in accord with the assumption made in this paper that there is some inherent factor in the plant which brings about the proper development of /

of the sexual tissues. For the genus Rhododendron it has been shown that the proper development of the sexual tissues may be upset by unseasonable high temperatures which affect the general physiology and well-being of the plant. It has also been shown that this "upset" of the course of development is expressed in a hastening or a retarding of tissue development with a consequent change in the time-interval between the reduction divisions. It has also been suggested that according as the hastening or the retarding of the development of the tissues is more evident in the male or female tissues, so the resulting abnormality is seen in the male or female tissues respectively; and, where both tissues are affected then abnormalities appear in both tissues. More evidence, however, is needed before this suggestion can be made a definite statement, also the facts with regard to protogynous hermaphrodite flowers and unisexual flowers must be obtained in fuller detail although SCHAFFNER (37, 38) has already shown that the normal sex expected in dioecious plants of known origin can be altered owing to abnormal changes having been made in the environment of the plant such as growing the plants out of season (mid-winter) and under greenhouse conditions.

(iii)/

(iii) SUMMARY:

The points which have been brought out in this discussion for hermaphrodite protandrous flowers may be briefly summarised thus: -

(a) The time required for the development of the sexual tissues is, in the majority of cases, correlated with the seasonal habit of the plant and may extend over two growing periods or be contained within one growing season.

(b) In hermaphrodite protandrous flowers the female tissues require a longer time from their initiation to their maturity than the male tissues.

(c) A longer or shorter dormant period of the sexual tissues occurs during the cycle of their development.

(d) The condition of the male and female tissues at the onset of the respective dormant periods varies according to the plant, the pollen-mother-cells or the pollen-tetrads being the usual condition for the male tissues and the fully developed placentae with or without indications of ovule rudiments being usually that of the female tissues.

(e) There is a predominantly male phase and a predominantly female phase in the cycle of development of the sexual tissues, the reduction divisions forming the boundary lines of these phases.

(f) In certain cases the reduction divisions tend to coincide, thereby lessening the time-interval between them and tending to make the male and female phases proceed simultaneously.

(g) This alteration of the normal time-interval between the reduction divisions is not affected by: -

i. The growth of other parts of the plant.

ii. The food supply.

iii. The size and number of the flowers produced.

iv. Low temperatures.

(h) This time-interval between the reduction divisions may be affected and, as a result, lessened, by unseasonable high temperatures.

(i) Unseasonable high temperatures bring about a hastening of the development of the sexual tissues.

(j) Hastening or retarding of the development of the sexual tissues causes maturity to be attained unnaturally and some consequent abnormality to be seen in the flower.

(k) Such abnormalities are of the nature of sterile tendencies on the part of the male or female tissues.

(l) Possibly the extent to which the "upset" of the course of development has affected the male and female tissues will determine the degree of sterility evident in the sexual tissues of the mature flower.

(m) The conditions during the cycle of development of the sexual tissues in hermaphrodite protogynous flowers has yet to be investigated.

VIII. CONCLUSIONS.

VIII. CONCLUSIONS:

From a comprehensive survey of the facts brought forward in this study certain general conclusions may be stated for the majority of members of the genus Rhododendron:

(a) A definite interval of time elapses between the flowering period of one year and the attainment of any stage in the development of the floral organs for the succeeding year. This interval varies according to the species and is longer in the case of the spring-flowering species than in the summer-flowering species. It extends over two growing periods.

But this definite interval of time may be altered, e.g., R. Keysii.

(b) At the onset of the winter period the stages reached in the male and female tissues of the majority of species is the same - the pollen-tetrad stage for the male tissues and placentae fully formed with ovular constrictions showing for the female tissues.

But no winter dormant period may occur, e.g., R. Keysii, although the male tissues may still have a brief dormant period.

(c) The male tissues in the bud develop more rapidly than the female in all species.

(d) There is a correlation between the rates of development of the male and female tissues - certain definite stages in the one set of tissues corresponding to certain definite stages in the other set of tissues - and this for all species bearing normal flowered inflorescences.

(e) The flower-buds pass through a period of more or less complete dormancy during the winter period. Even when there is no winter /

winter period, e.g., R.Keysii or when the external conditions are such that winter conditions are ameliorated as in the species grown under glass, a dormant period, of the male tissues at least, is still included in the cycle of development.

(f) There is a definite interval of time elapsing between the occurrence of the male and female reduction divisions:

But this interval of time may be altered, e.g., R. Keysii.

(g) The interval of time between the reduction divisions is affected by unseasonable high temperatures influencing the development of the sexual tissues.

With the facts deduced from the study of these species of Rhododendron taken in conjunction with the investigations carried out by other workers on various different plants, the writer of this paper arrived at the following general conclusions: -

In any hermaphrodite protandrous flower: -

(a) there is a definite interval of time normally elapsing between the male and female reduction divisions;

(b) the relation of the male and female reduction divisions in time has a determining effect on the sexual efficiency of the gametes;

(c) this time relation may be altered by certain external conditions interacting with the inherent nature of the plant.

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49. *Wilson, E.H., & Rehder, A.*, 1921. A Monograph of Azaleas.

X. FIGURES.

LIST of ABBREVIATIONS used in FIGURES.

A.W. = Wall of Anther.
T.L. = Tapetal Layer.
Sp.T. = Sporogenous Tissue.
P.M.C. = Pollen Mother Cells.
P.S. = Pollen Sac.
P.T. = Pollen Tetrads.
P. Tubes = Pollen Tubes.
G.W. = Wall of Gynoecium.
C.T. = Central Tissue of Gynoecium.
C.C. = Central Cavity of Style.
L.W. = Wall of Loculus.
L.S. = Cavity of Loculus.
Pl. = Placenta.
Ov.R. = Immature Ovules.
Arch.C. = Archesporial Cell.
E.S. = Embryo Sac.
Emb. = Embryo.
Susp. C. = Suspensor Cells.
Integ. = Integument.
Ov. = Mature Ovules.
Ov. W. = Wall of Ovule.
Tn. = Cells filled with Tannin.
V.B. = Vascular Tissue.

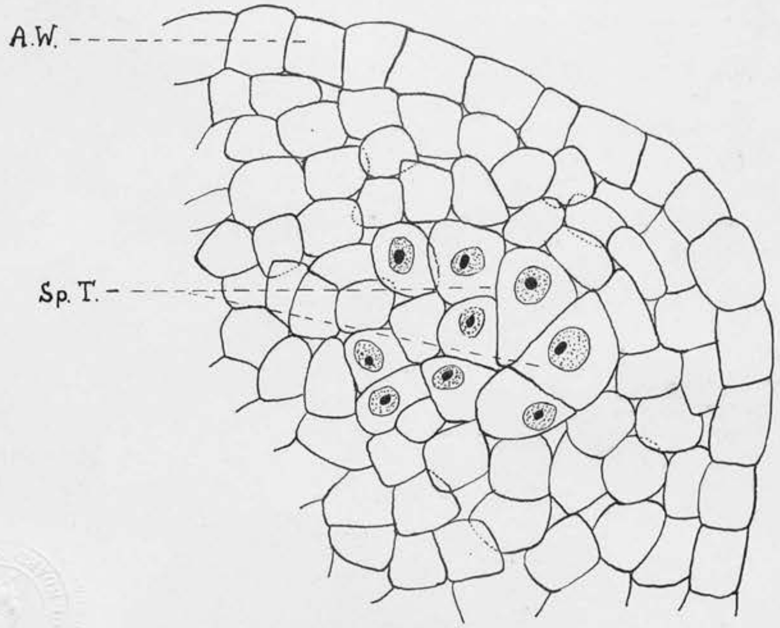

Fig. I. *R. Yunnanense*. T.S.Pollen Sac. collected 22/8/30.
10μ, x 1000.

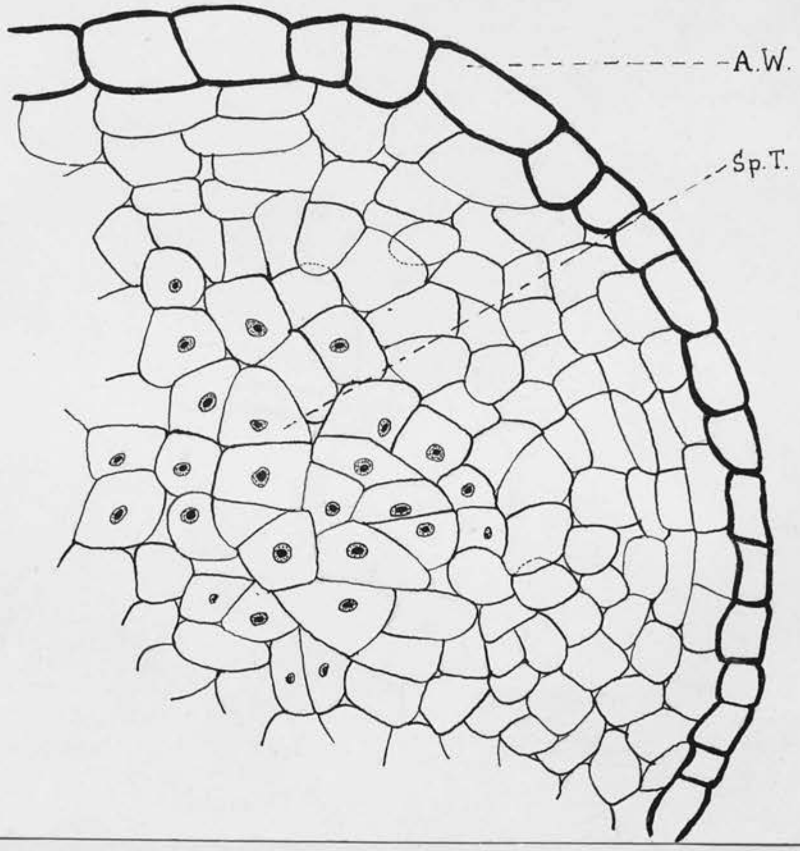

Fig. II. *R. Yunnanense*. T.S.Pollen Sac. collected 1/9/30.
5μ, x 1000.

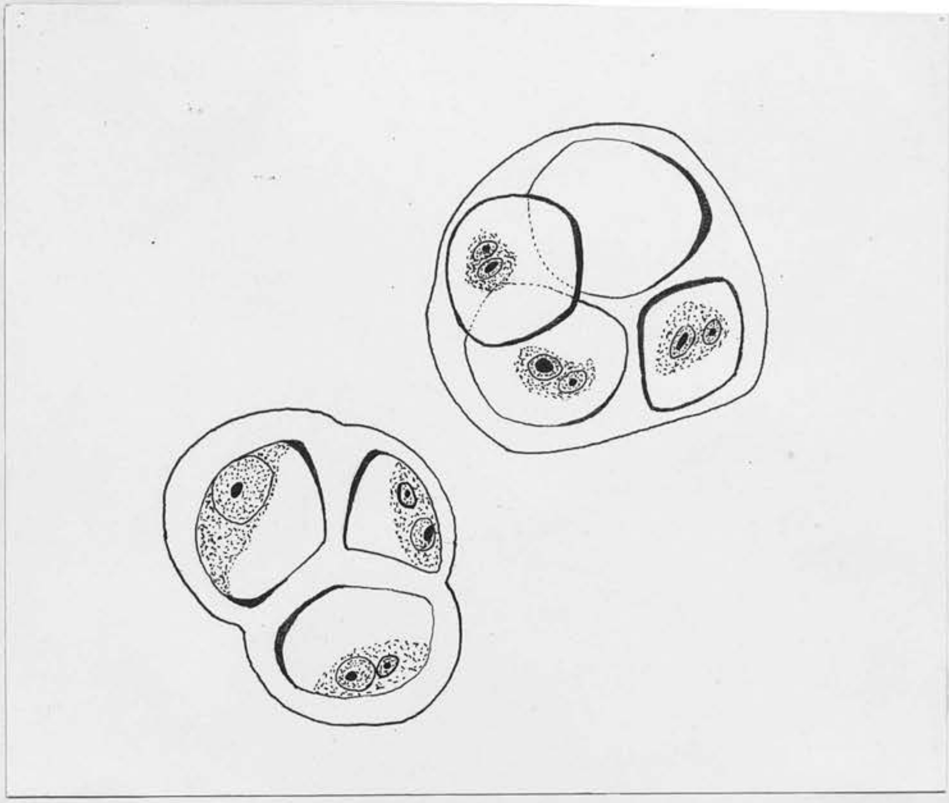

Fig. III. R. Yunnanense: Pollen Tetrads, collected 2/3/31 x 1000.

Fig. IV. R. Yunnanense. T.S.Pollen Sac. collected 3/4/31. 8 μ, x 250.

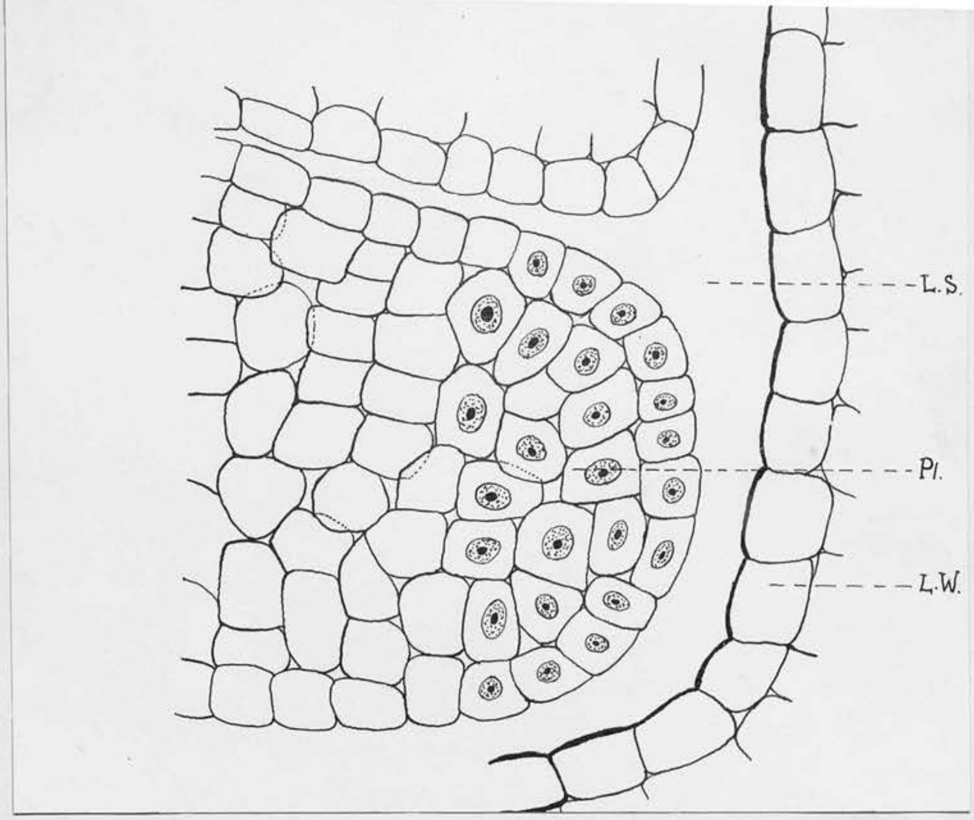

Fig. V. R. Yunnanense: T.S.Loculus. collected 22/8/30.
10 μ. x 1000.

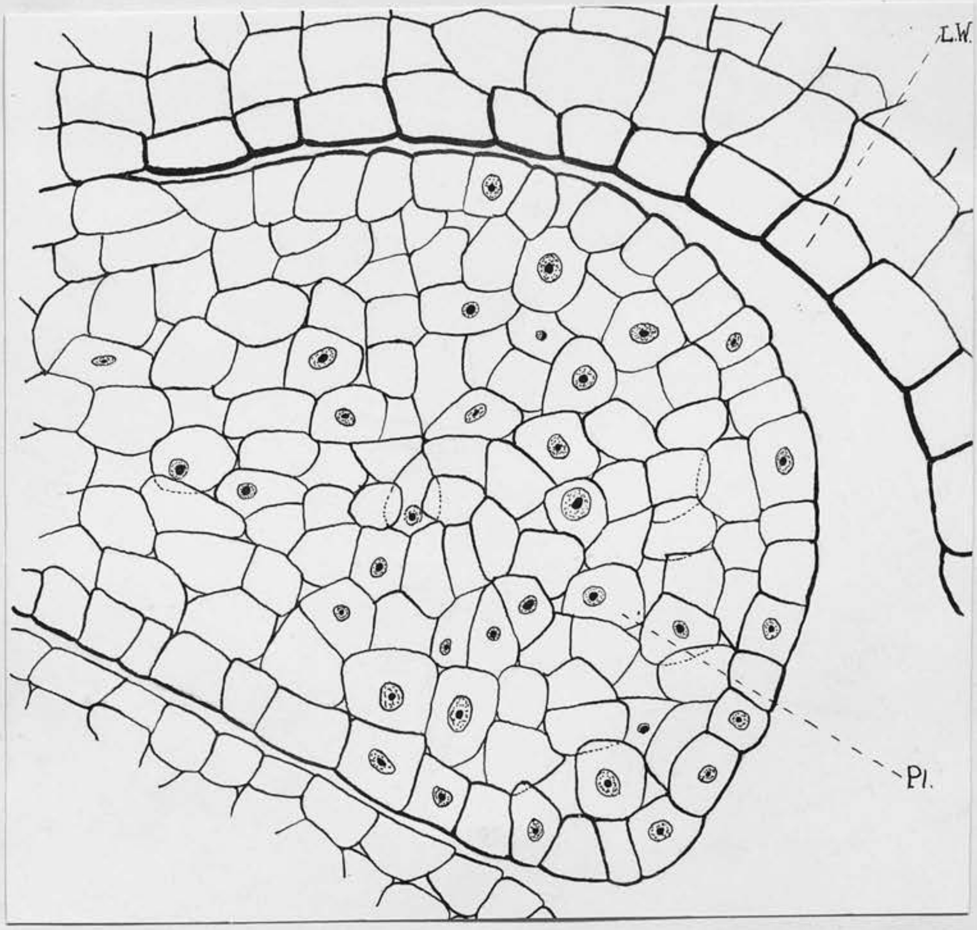

Fig. VI. R. Yunnanense: T.S.Loculus: collected 1/9/30. 5 μ.
x 1000.

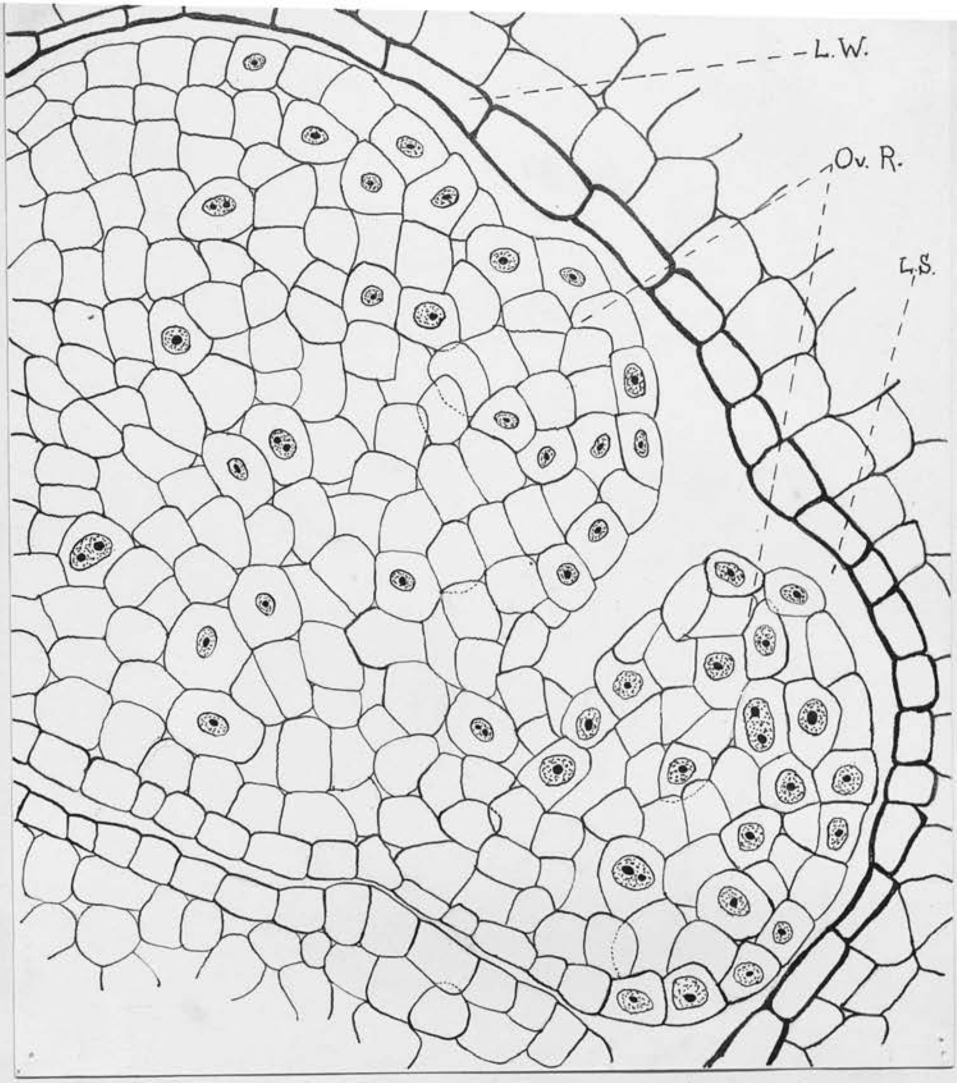

Fig. VII. R. Yunnanense: T.S.Loculus. collected 2/3/31.
8 µ, x 750.

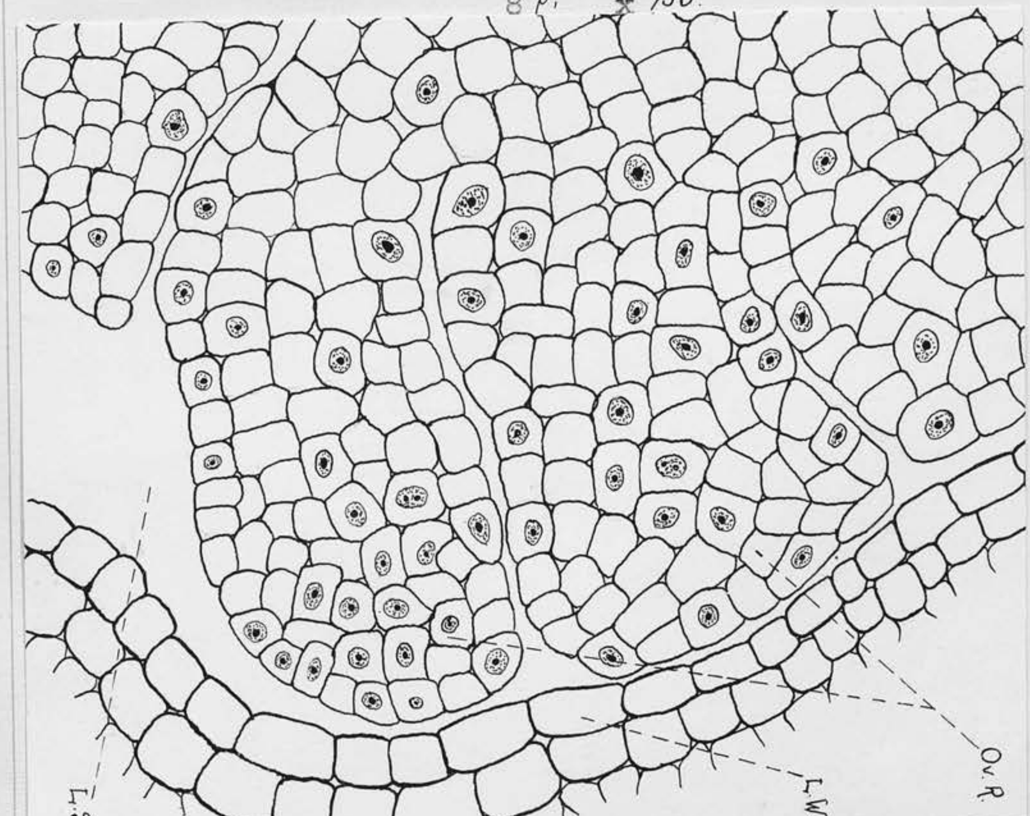

Fig. VIII. R.Yunnanense: T.S.Loculus. collected 17/3/31
8 µ, x 750.

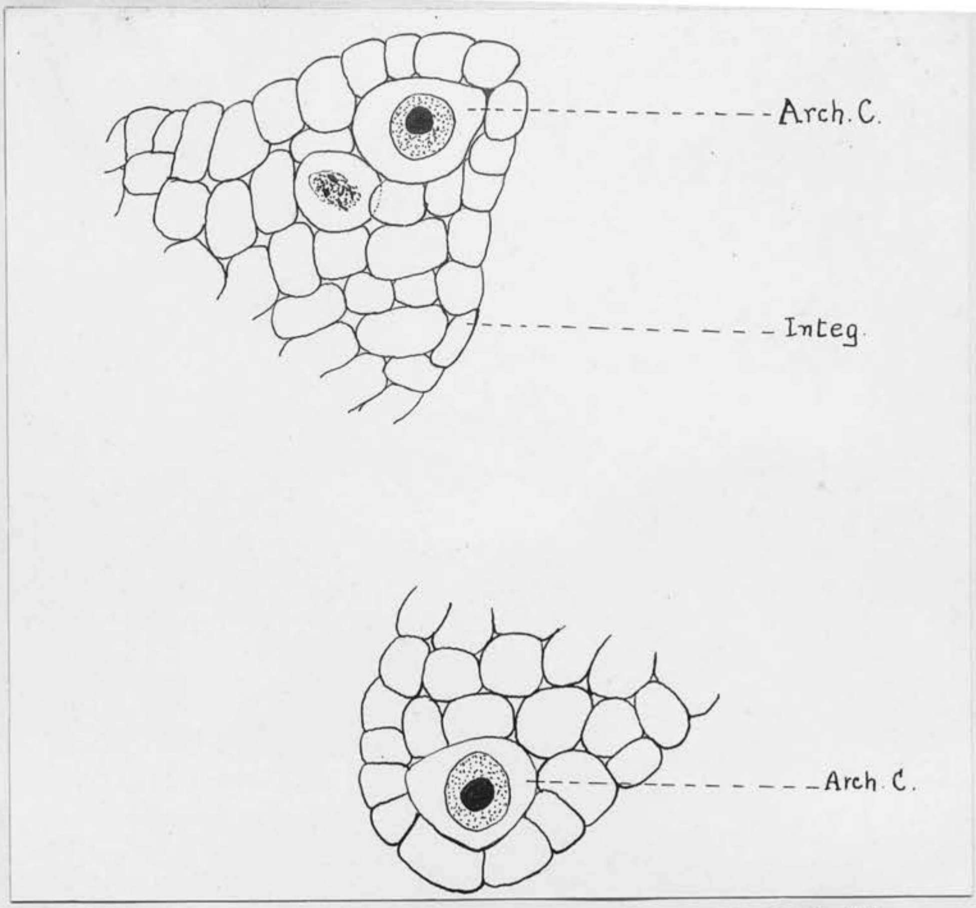

Fig. IX. R. Yunnanense: T.S. Ovules, collected 17/3/31.
8µ, x 1000.

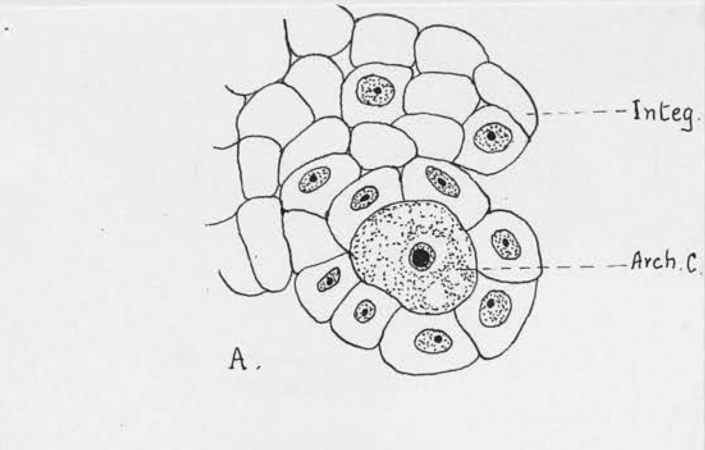

Fig. X. R. Yunnanense (glasshouse) (A) T.S., and (B) L.S. Ovules collected 17/3/31. 8µ, x 1000.

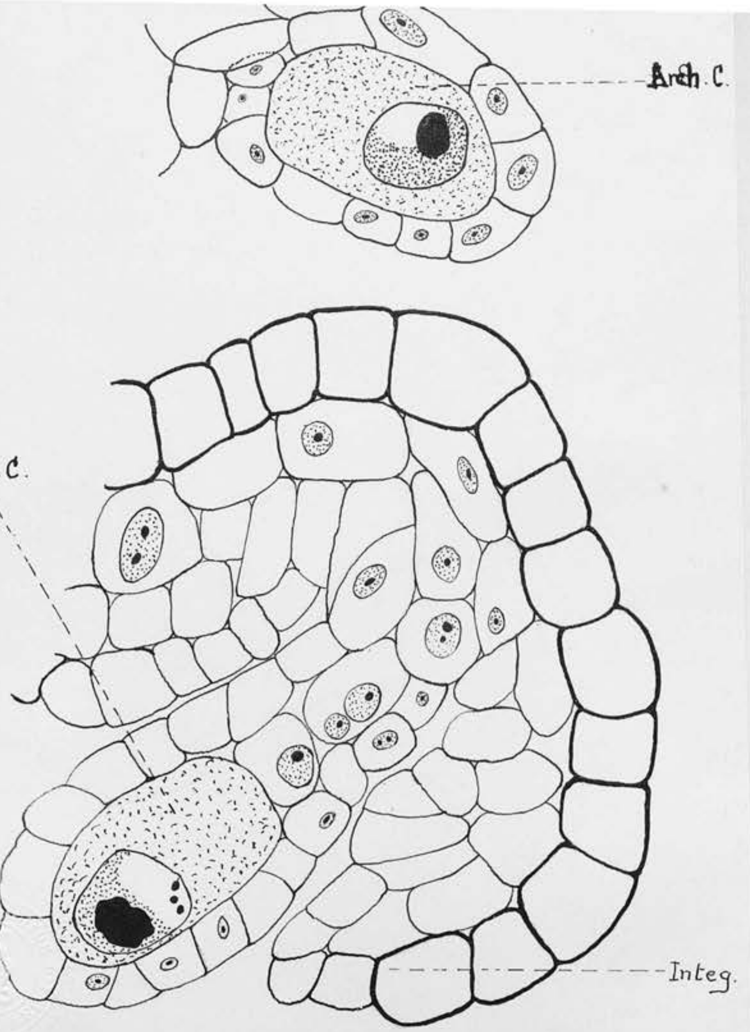

Fig. XI. R. Yunnanense: L.S.Ovules, collected 27/3/31.
8μ, x 1000.

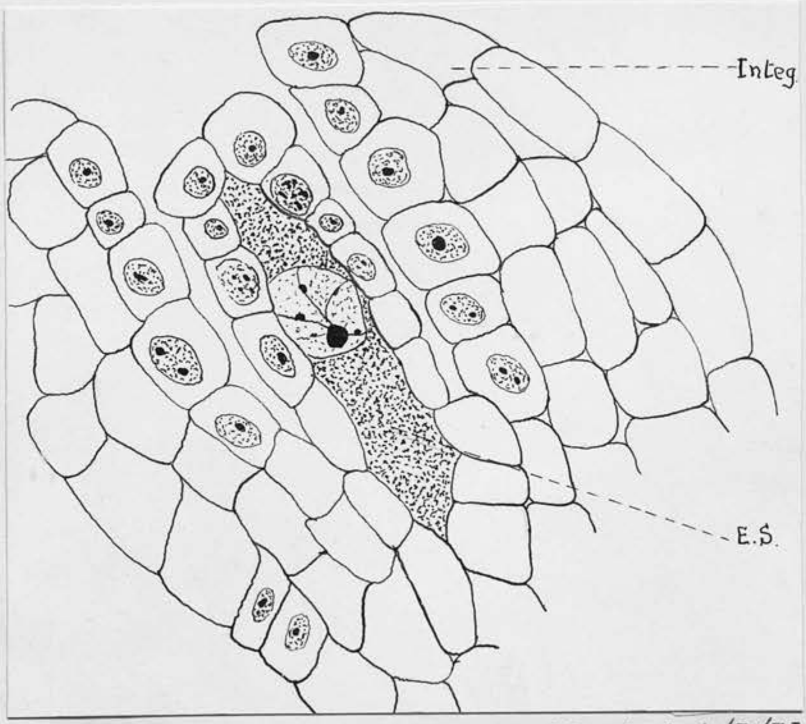

Fig. XII. R. Yunnanense: L.S.Ovule. collected 4/5/31.
8μ, x 1000.

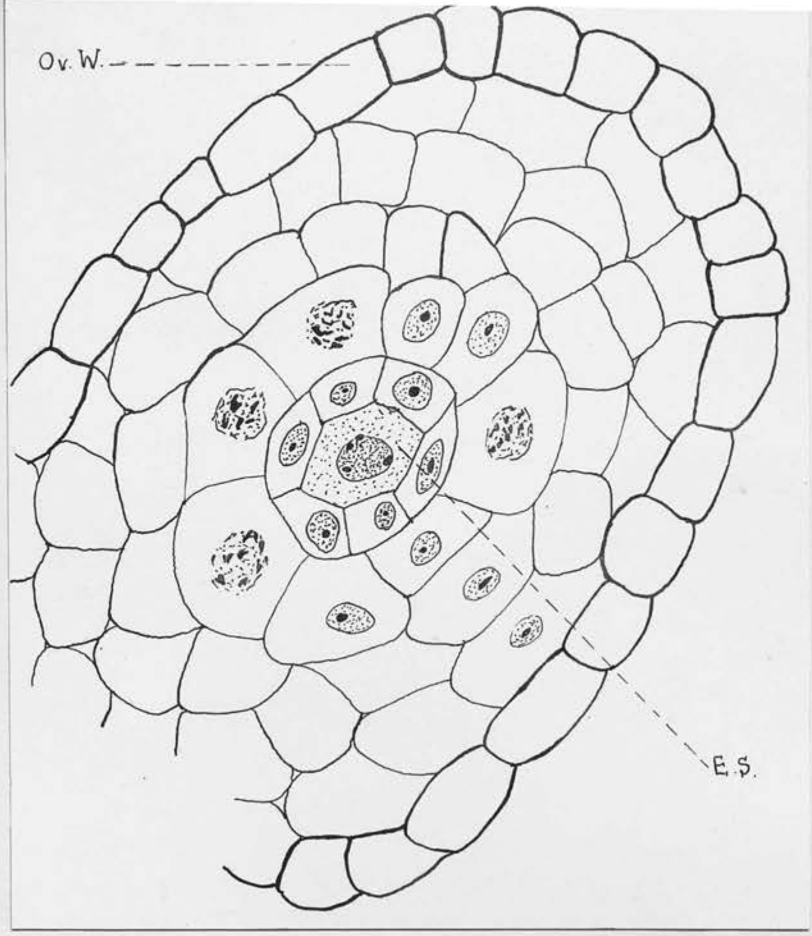

Fig. XIII. R. Yunnanense: T.S.Ovule, collected 4/5/31.
8 μ, x 1000.

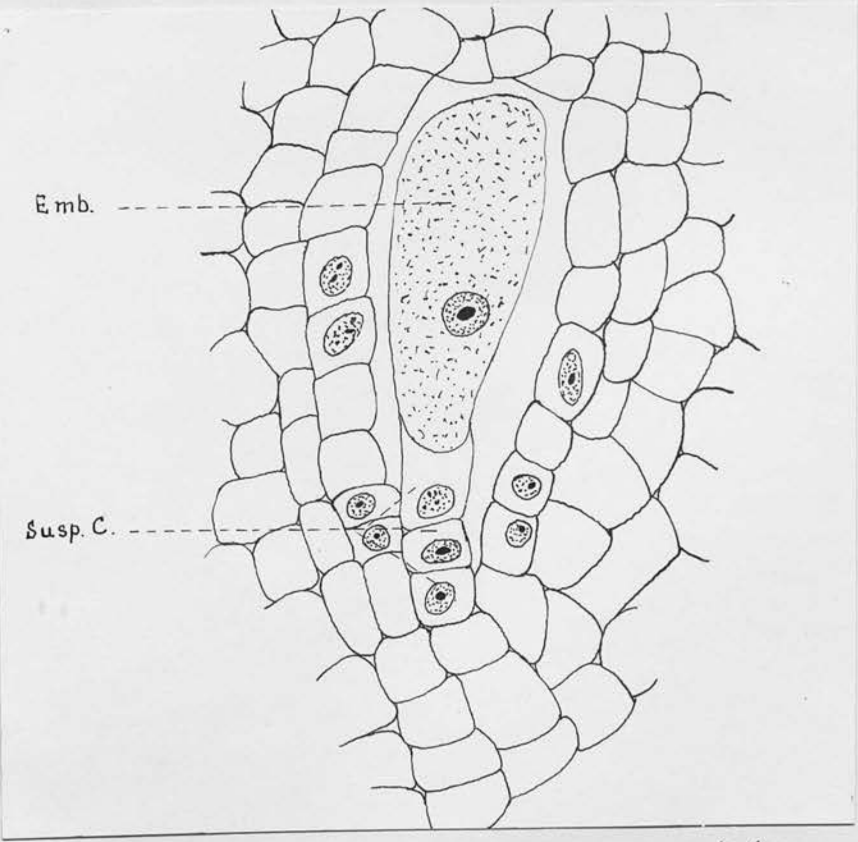

Fig. XIV. R. Yunnanense: L.S.Ovule. collected 8/5/31.
8 μ, x 1000.

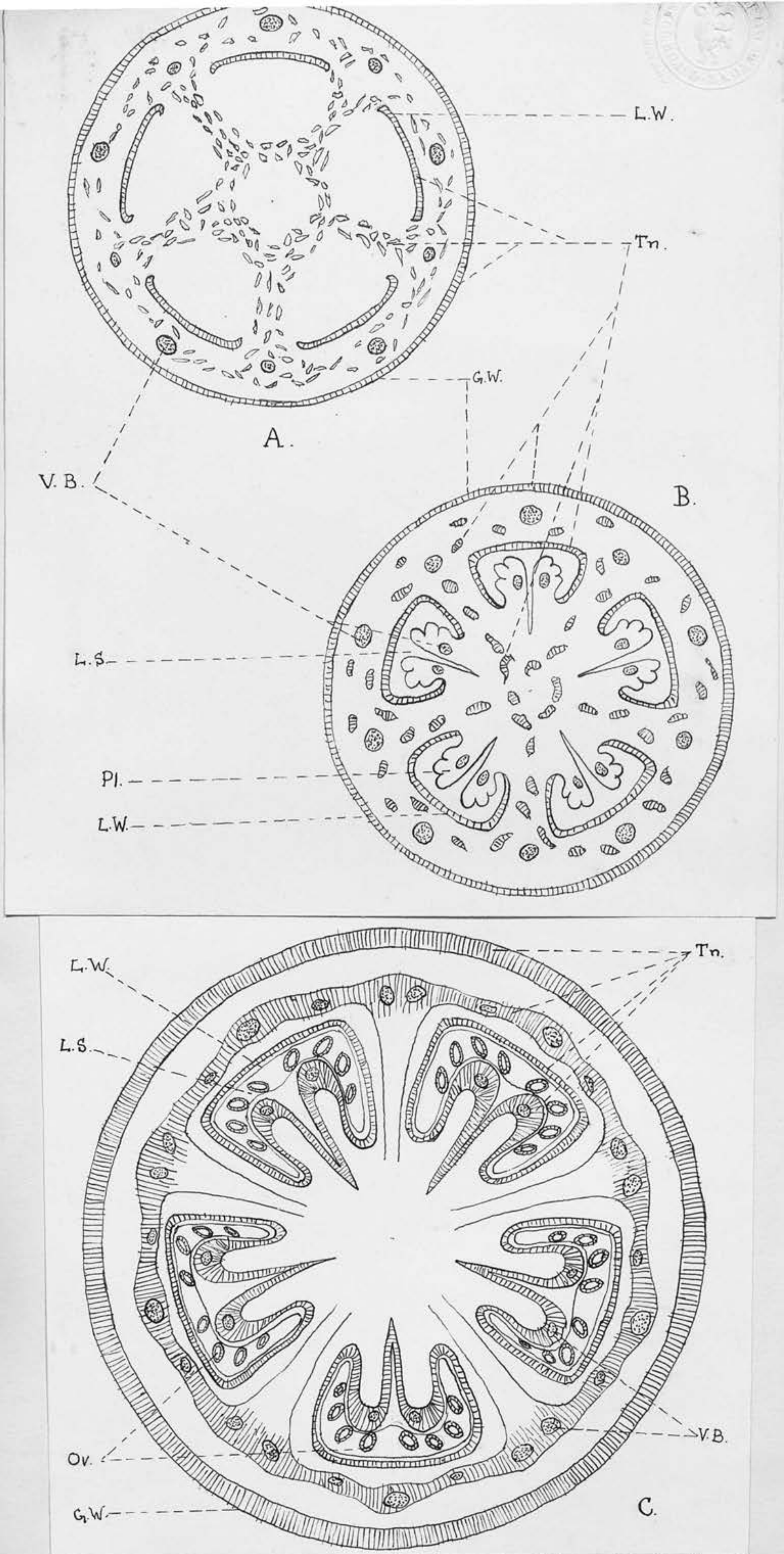

Fig. XV. R. Yunnanense: Diagrams illustrating distribution of Tannin and Vascular Tissue in Gynoecia of varying ages. A. collected 22/8/30; B. collected 27/3/31; C. collected 8/5/31.

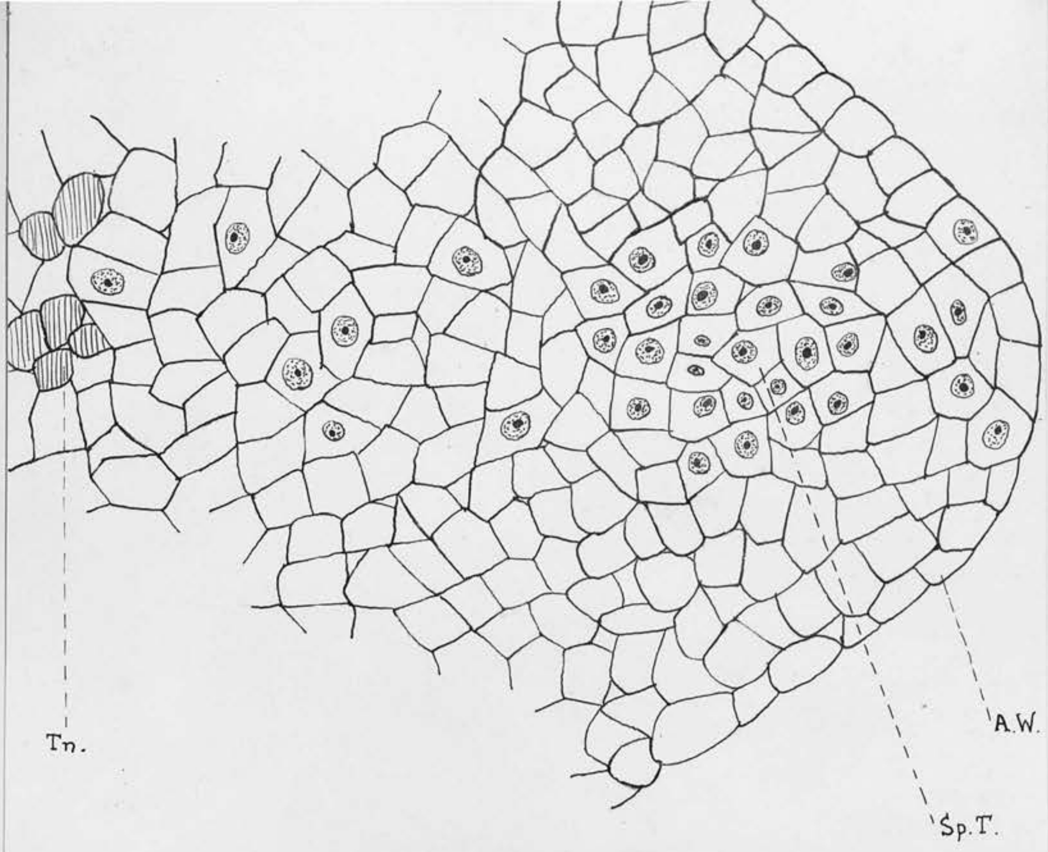

Fig. XVI. R. arboreum: T.S. Pollen Sac. collected 9/7/30.
10µ, x 1000.

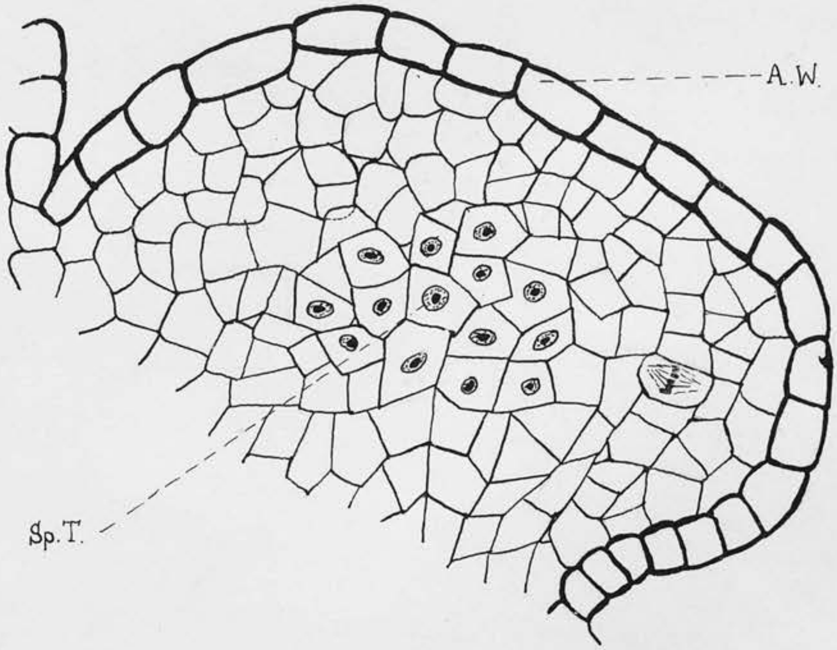

Fig. XVII. R. arboreum: T.S. Pollen Sac. collected 1/9/30.
10µ, x 1000.

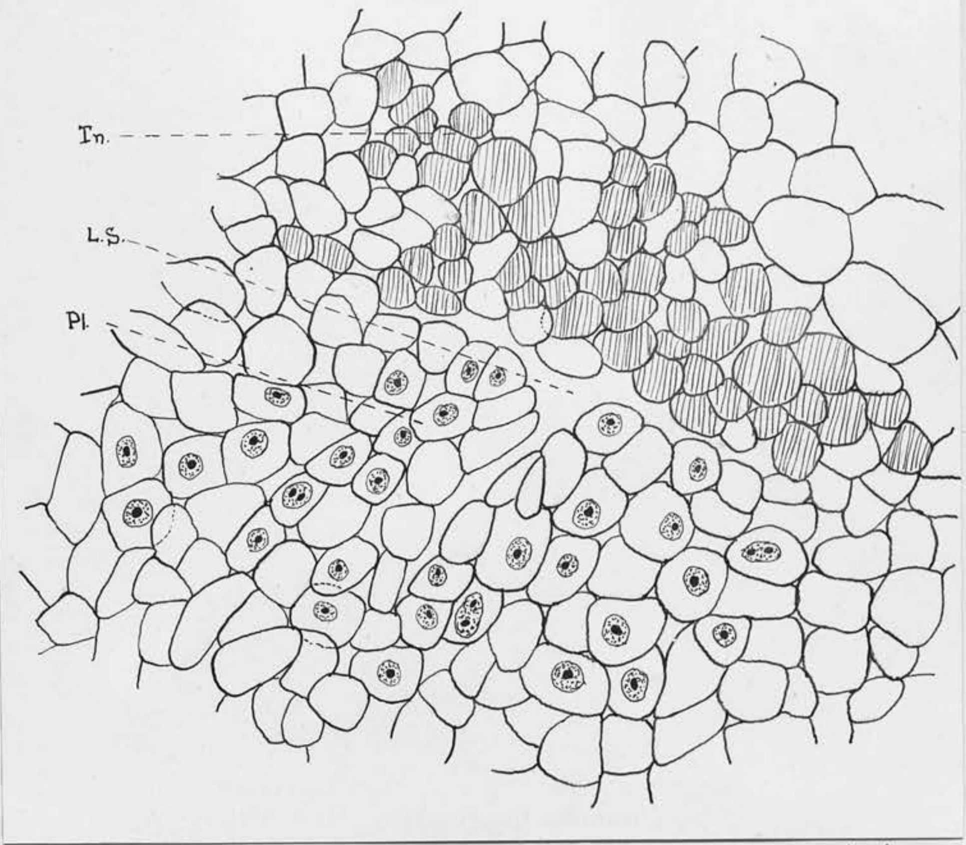

Fig. XVIII. R. arboreum: T.S.Loculus. collected 9/7/30.
10 μ, x 1000.

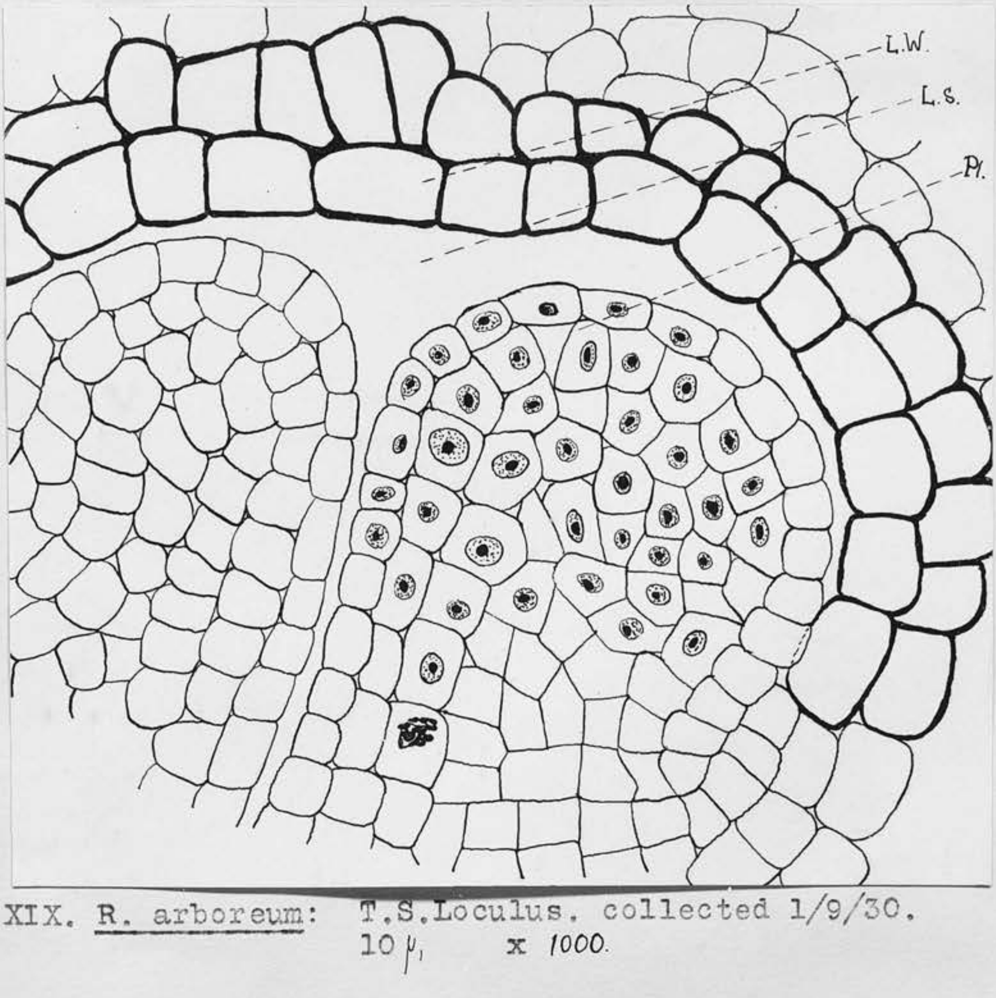

Fig. XIX. R. arboreum: T.S.Loculus. collected 1/9/30.
10 μ, x 1000.

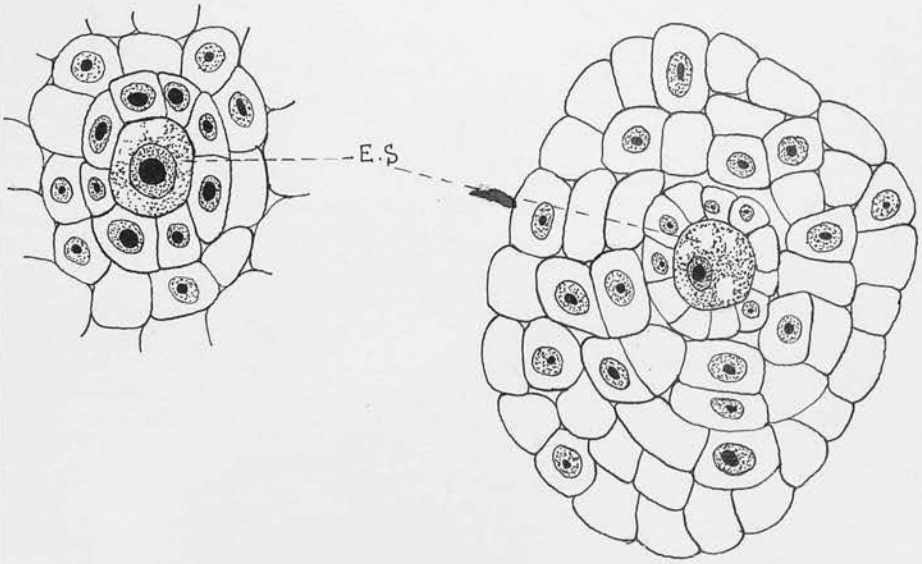

Fig. XX. R. arboreum: T.S.Ovules. collected 27/3/31. 8μ, x 1000.

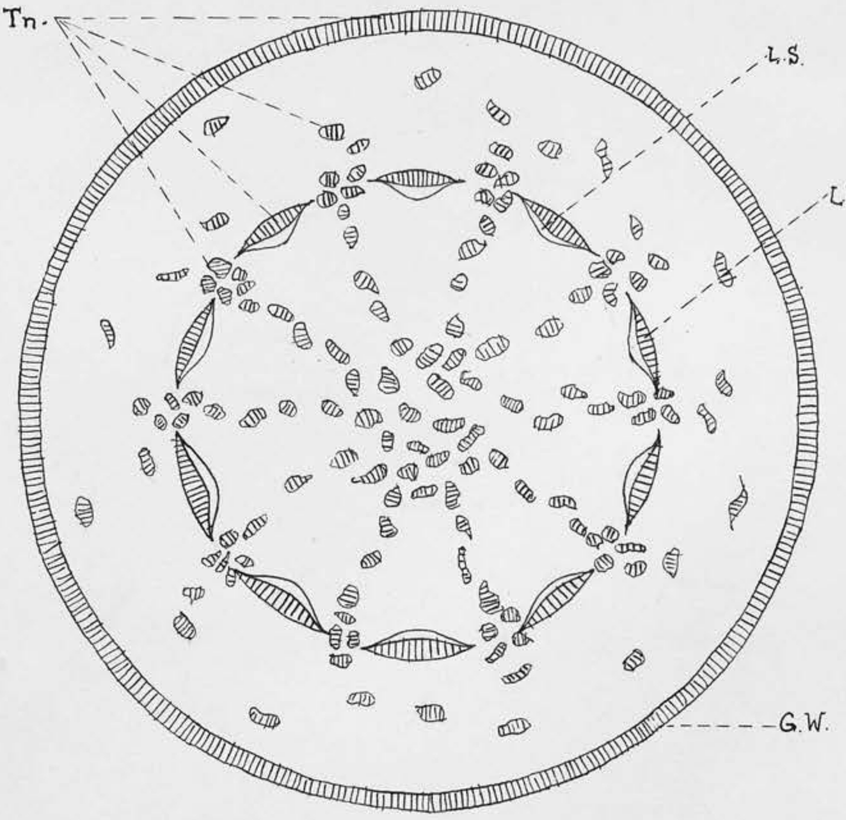

Fig. XXI. R. arboreum: Diagram showing distribution of Tannin in gynoecium collected 9/7/30.

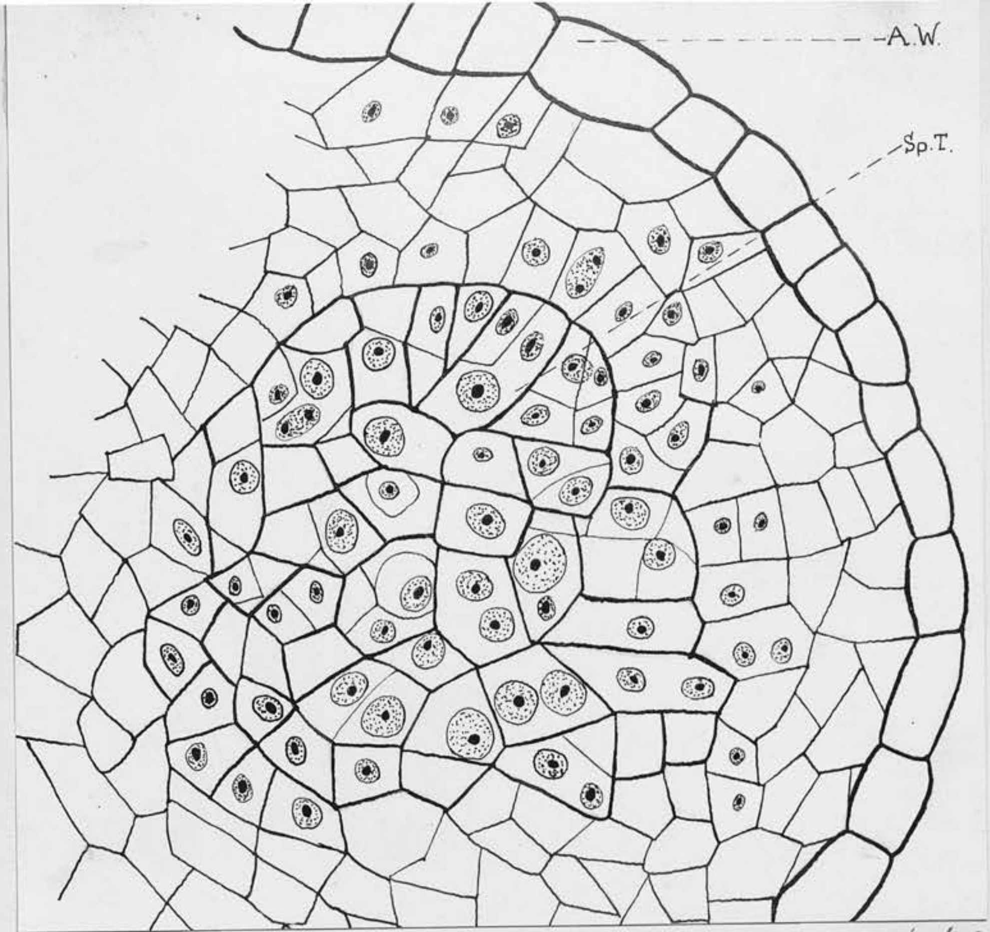

Fig. XXII. R. galactium: T.S.Pollen Sac. collected 11/7/30.
10µ, x 1000.

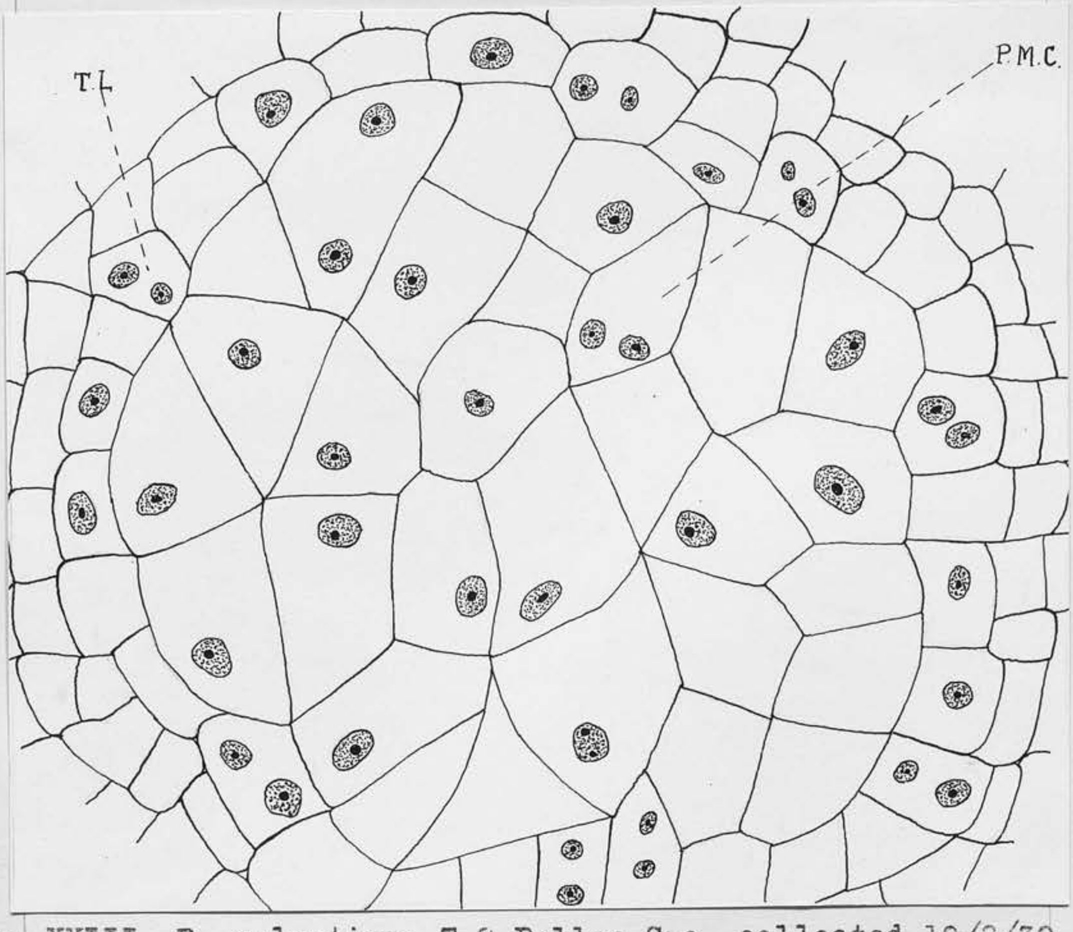

Fig. XXIII. R. galactium: T.S.Pollen Sac. collected 19/8/30.
8µ, x 1000.

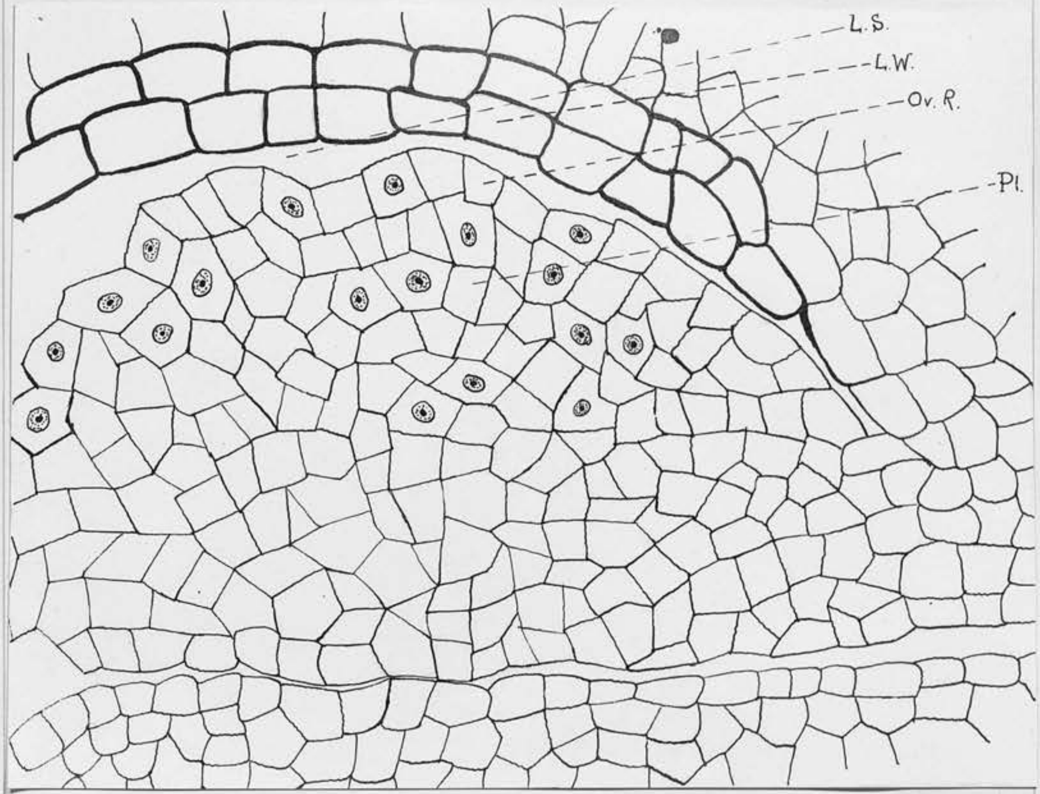

Fig. XXIV. R. galactium: T.S. Loculus, collected 11/7/30.
10 µ, x 1000.

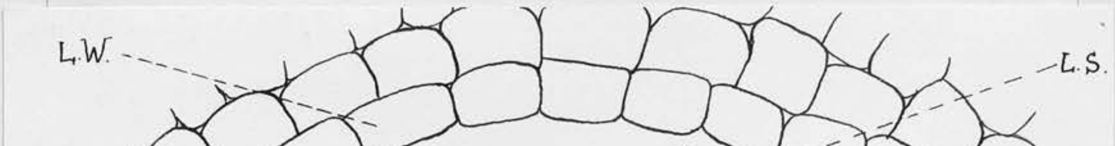

Fig. XXV. R. galactium: T.S. Loculus. collected 19/8/30.
8 µ, x 1000.

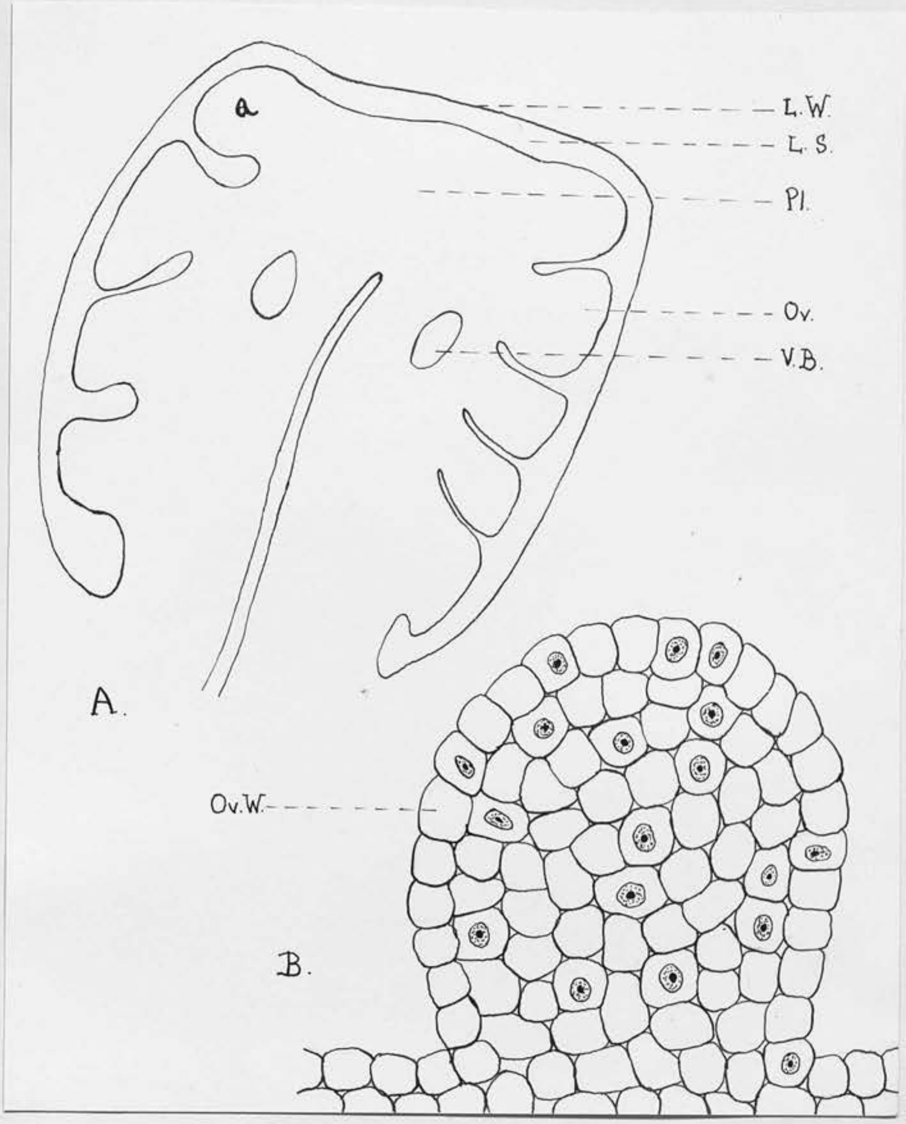

Fig. XXVI. R. galactium: A. T.S.Loculus x 250 collected, 17/3/31.
B. T.S.Single Ovule (a. above) 8μ. x 1000. collected 17/3/31.

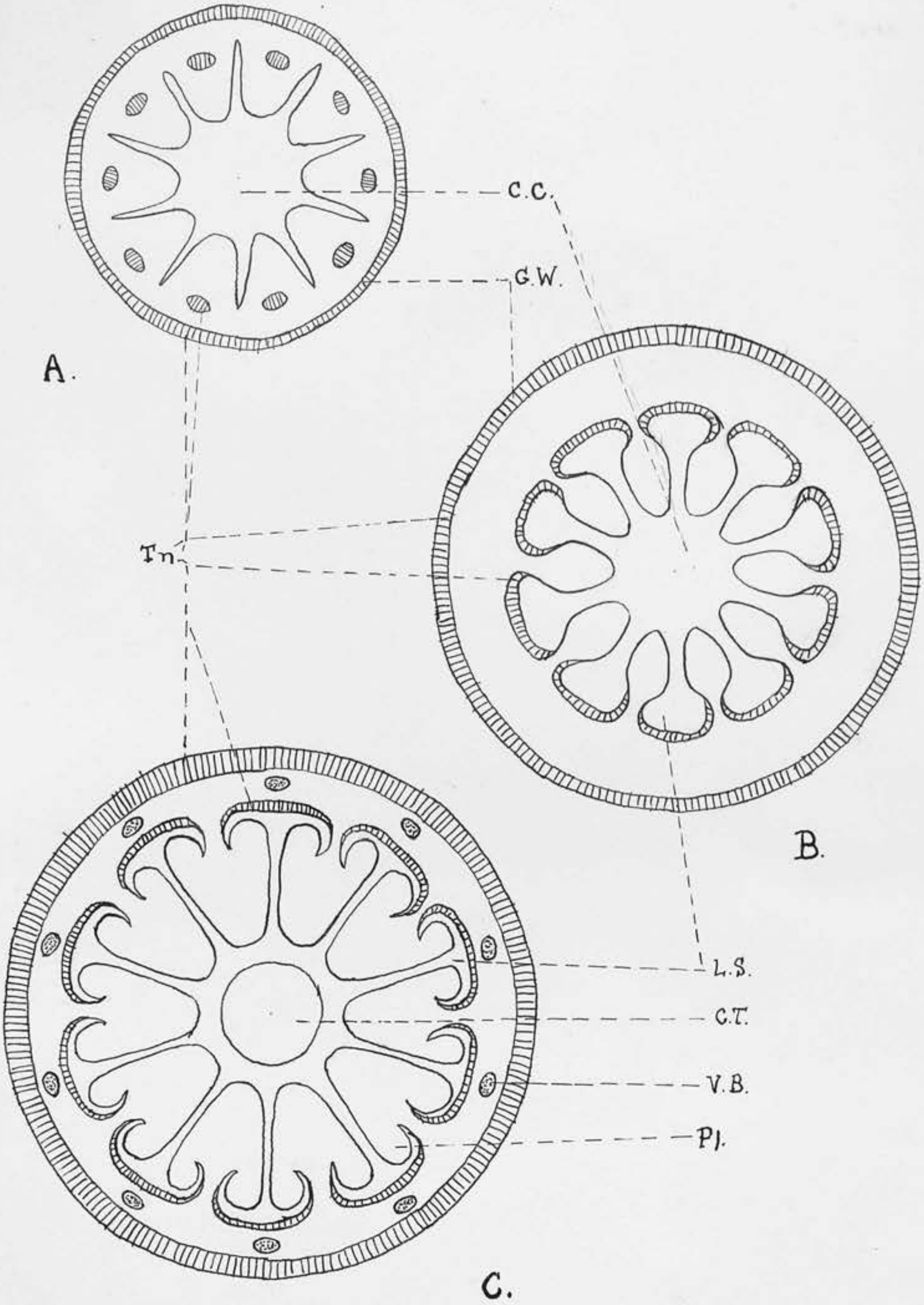

Fig. XXVII. R. galactium: T.S.Gynoecium (diag.) collected 9/7/30. to show relation of different tissues at varying levels. (P.T.O.)

A. T.S. Style.
B. T.S. towards base of Style.
C. T.S. upper part of Gynoecium.

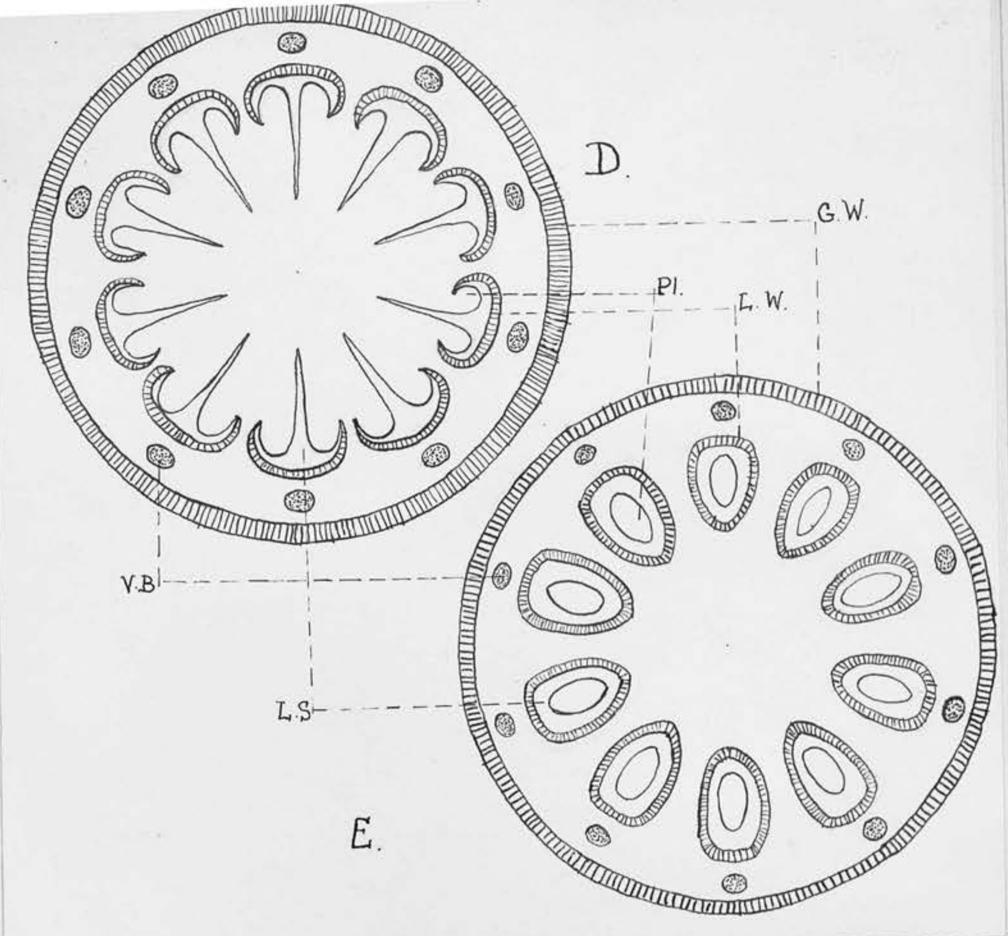

Fig. XXVII. R. galactium: D. T.S. centre of Gynoecium.
E. T.S. towards base of Gynoecium.

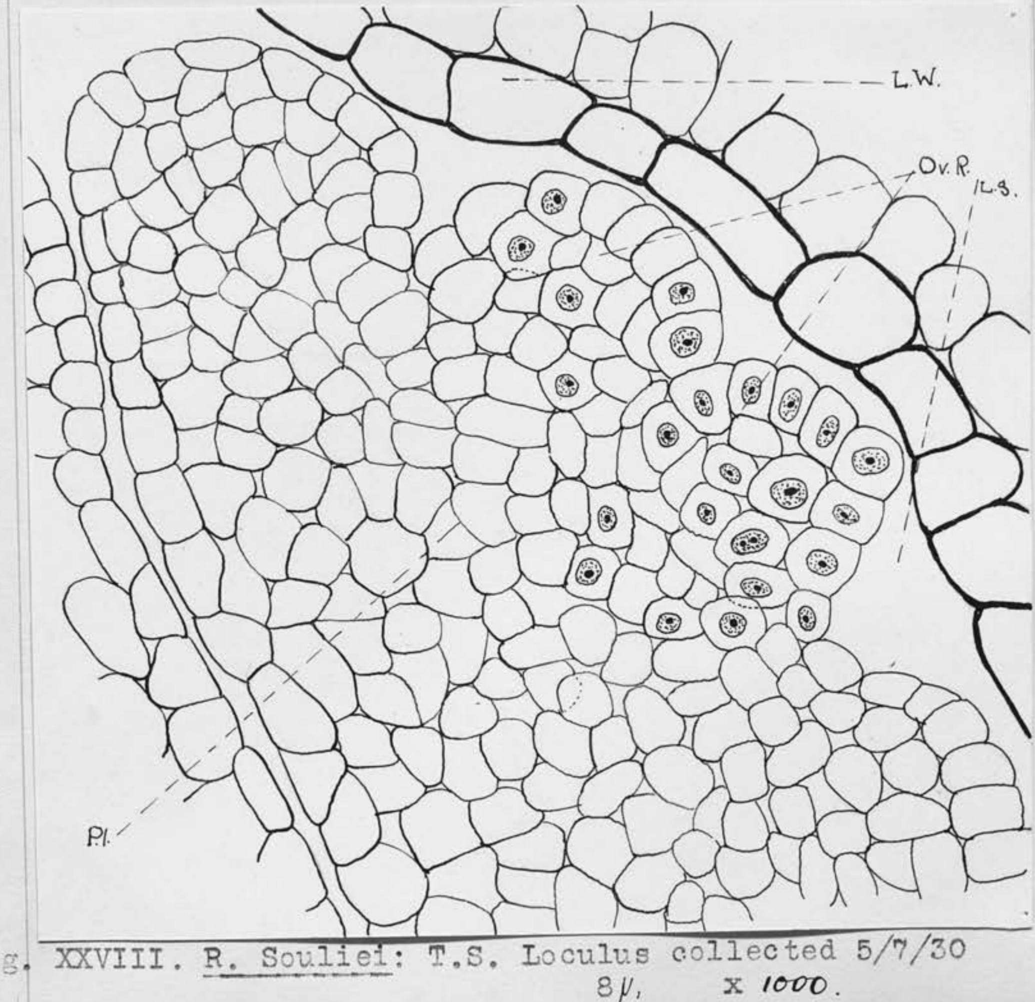

Fig. XXVIII. R. Souliei: T.S. Loculus collected 5/7/30
8µ, x 1000.

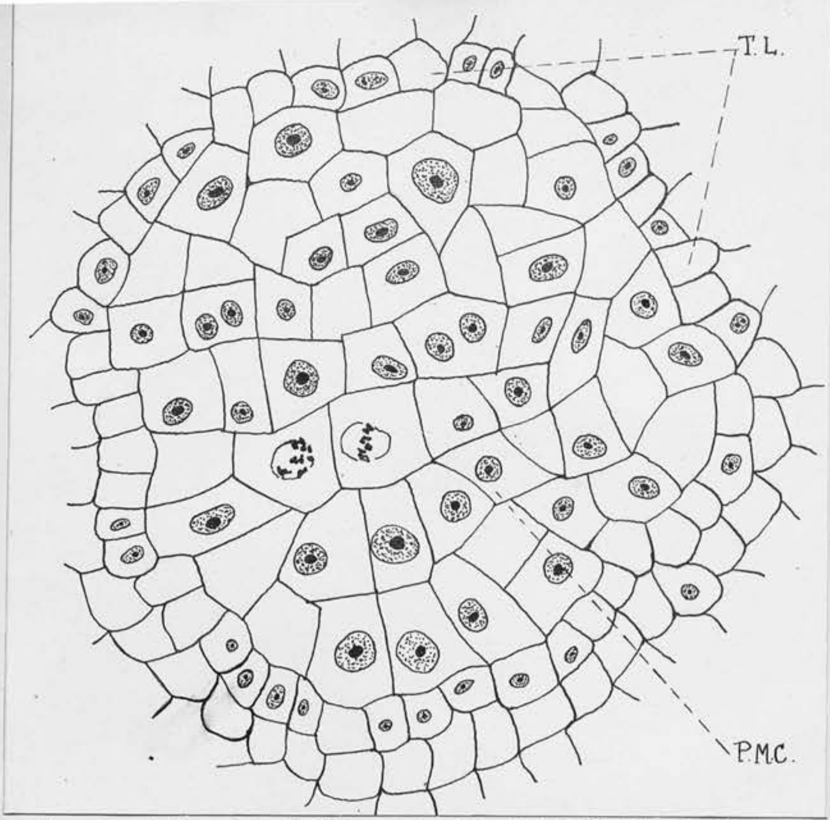

Fig. XXIX. R. Aucklandi X Thomsoni: T.S. Pollen Sac. collected 7/7/30. Saf. Gen. Viol. 10μ, x 1000.

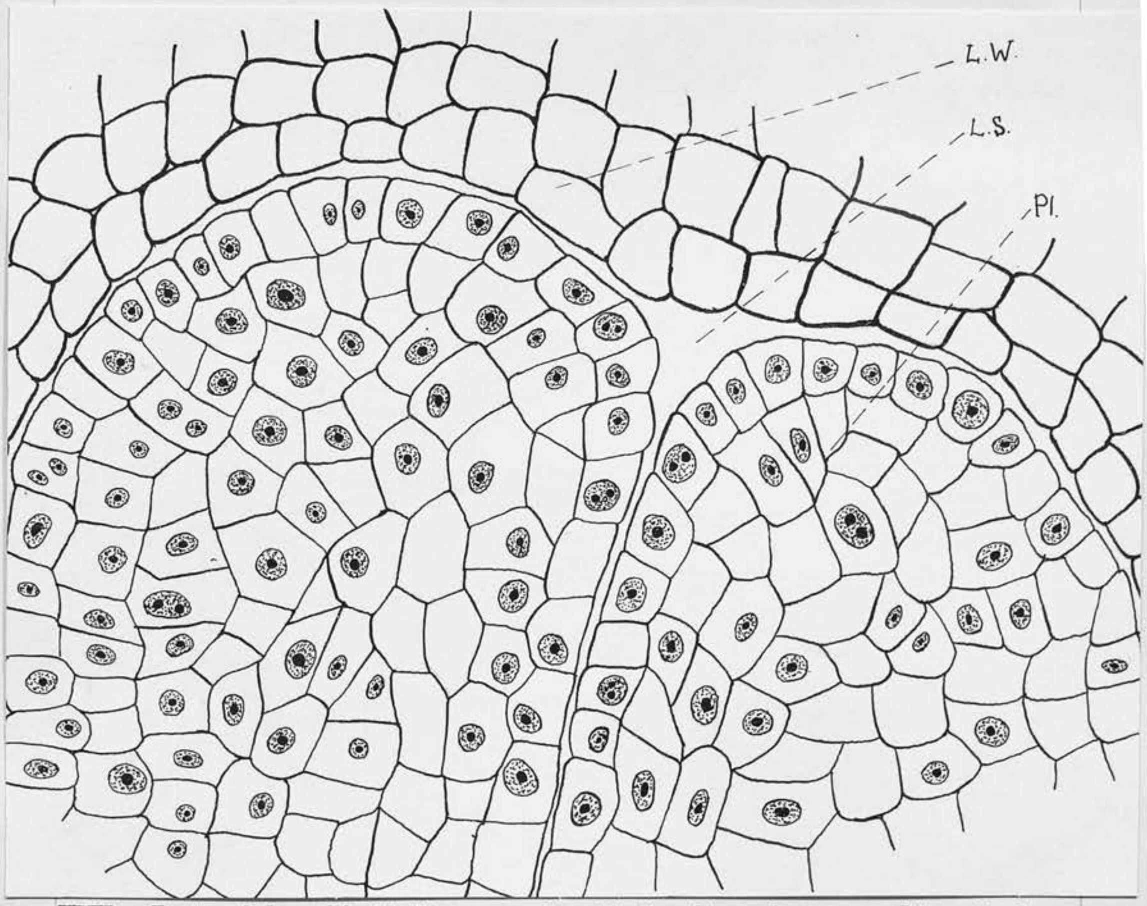

Fig. XXX. R. Aucklandi X Thomsoni: T.S. Loculus, collected 7/7/30. Saf. Gen. Viol. 10μ, x 1000.

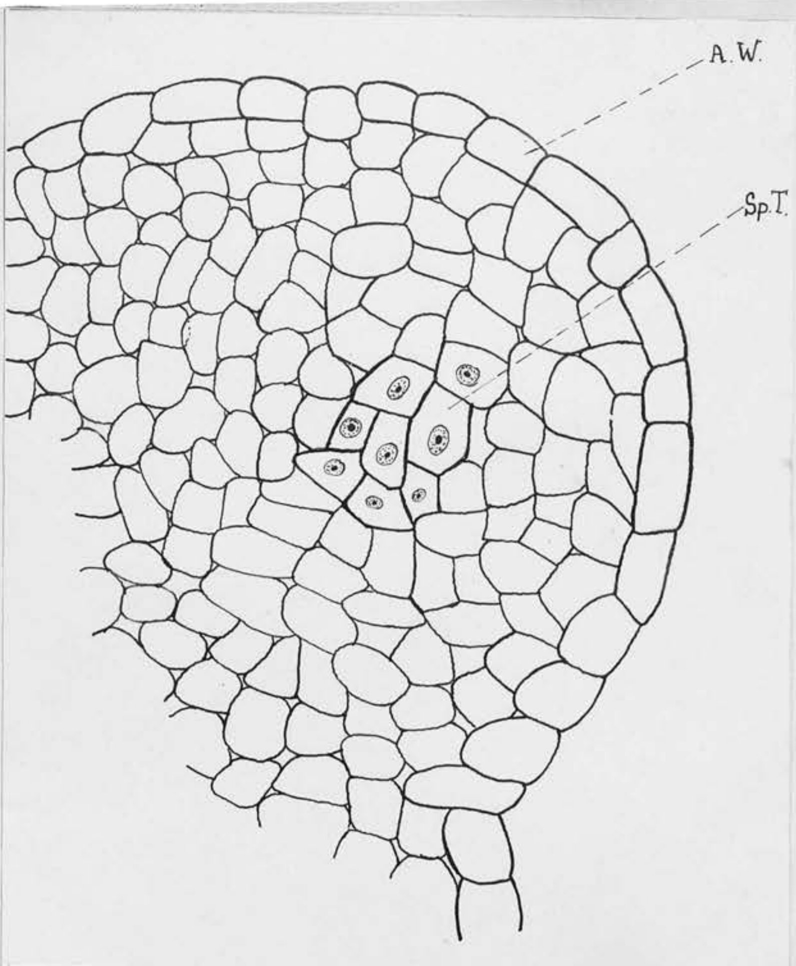

Fig. XXXI. R. arboreum-album: T.S.Pollen Sac. collected 8/7/30. 10 μ, x 1000.

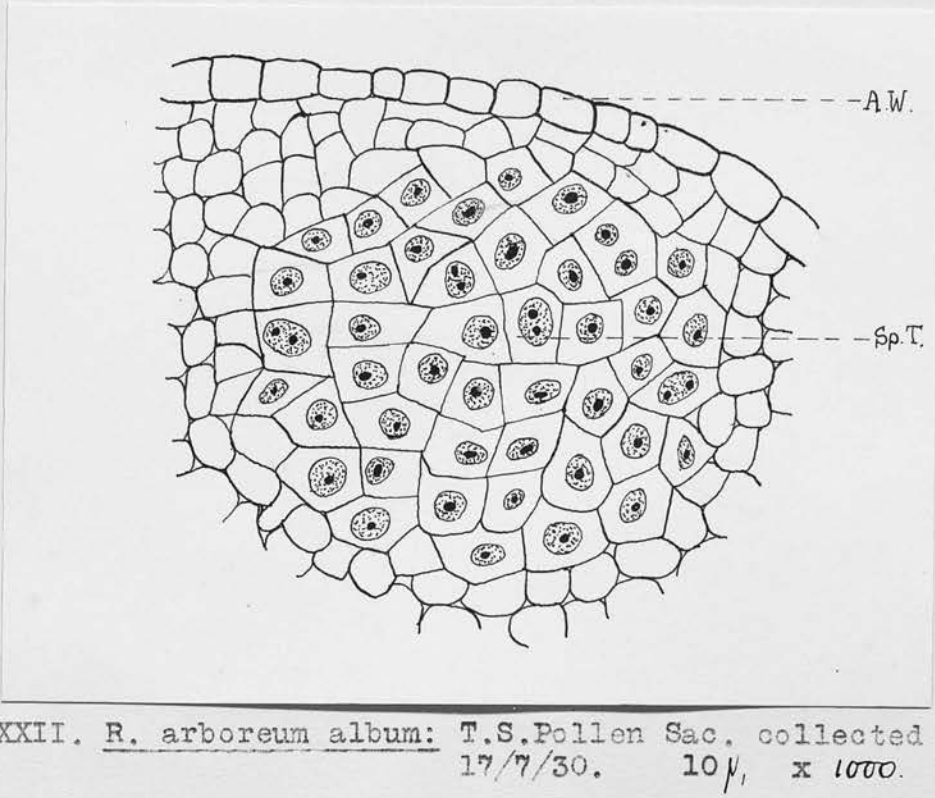

Fig. XXXII. R. arboreum album: T.S.Pollen Sac. collected 17/7/30. 10 μ, x 1000.

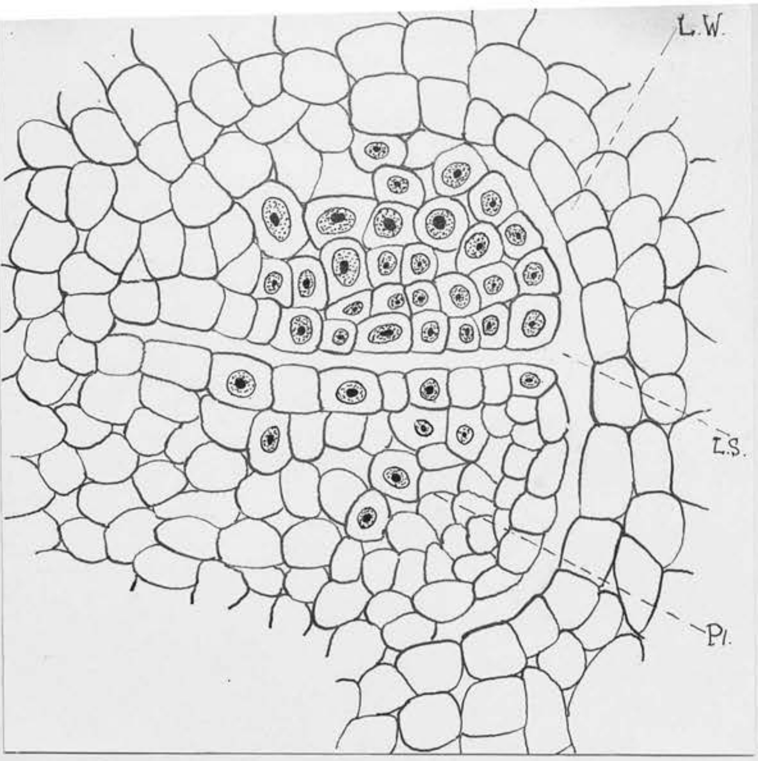

Fig. XXXIII. **R. arboreum album**: T.S. Loculus, collected 8/7/30. 10μ, x 1000.

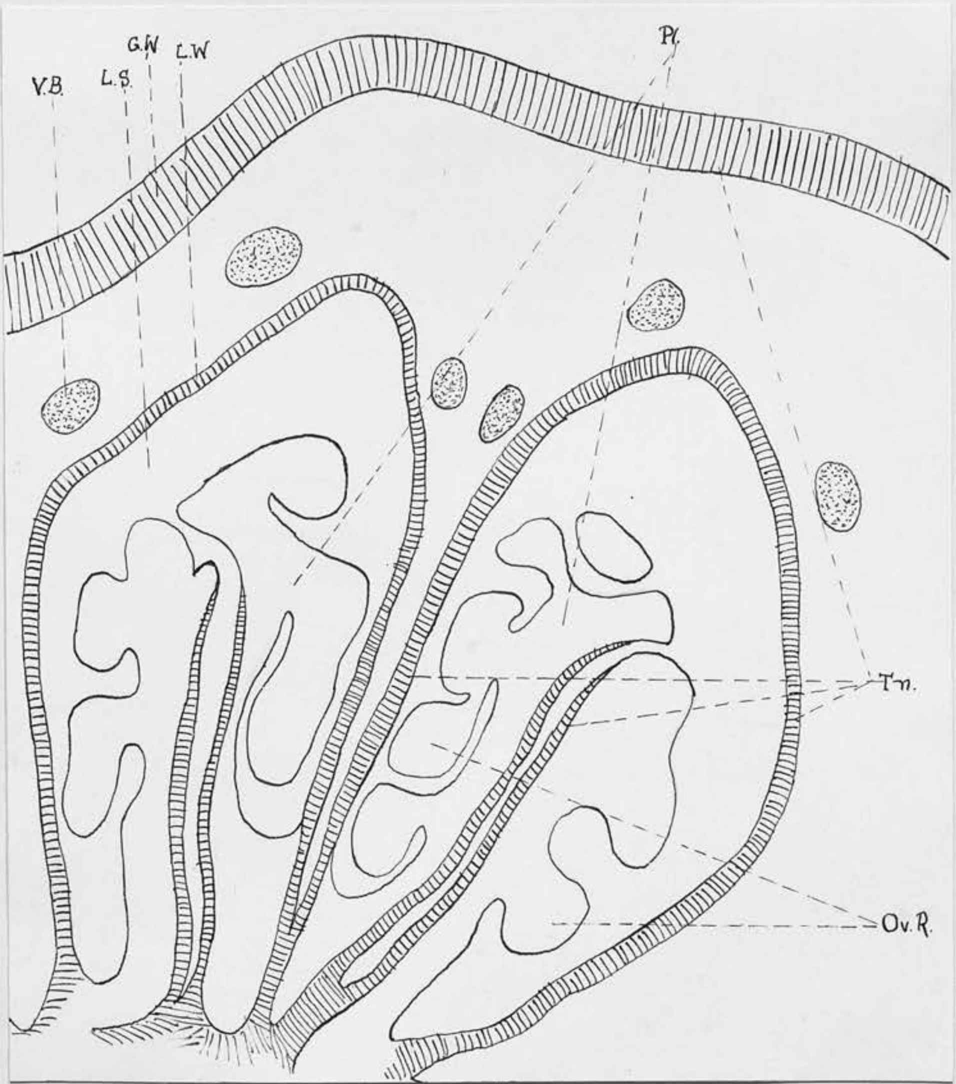

Fig. XXXIV. **R. arboreum album**: T.S. adj. Loculi (semi-diag) collected 12/3/31. 8μ, x 180.

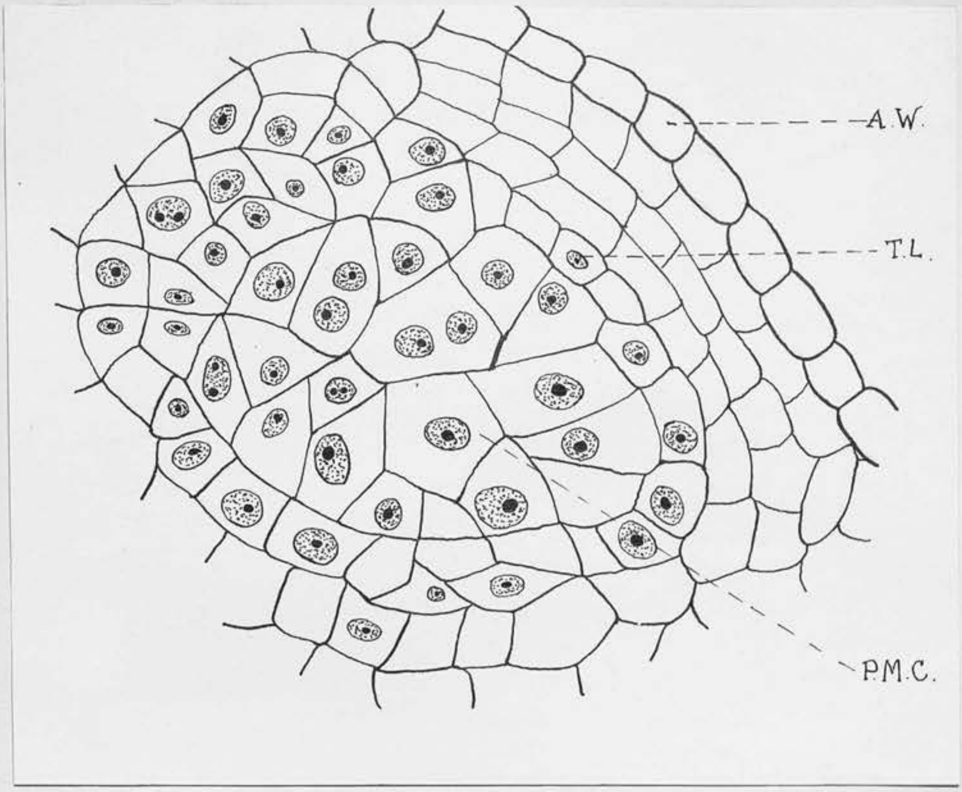

Fig. XXXV. R. brachyanthum: T.S. Pollen Sac. collected 8/10/31. 10 µ, x 1000.

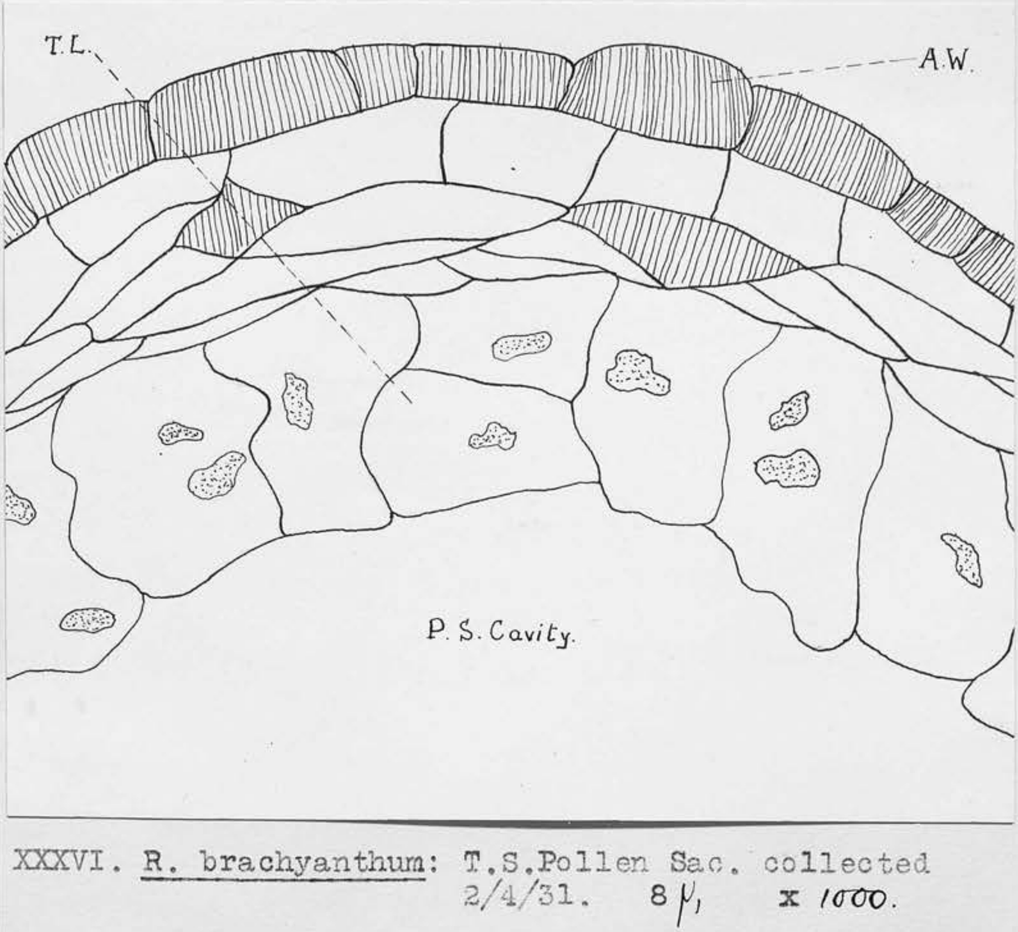

Fig. XXXVI. R. brachyanthum: T.S. Pollen Sac. collected 2/4/31. 8 µ, x 1000.

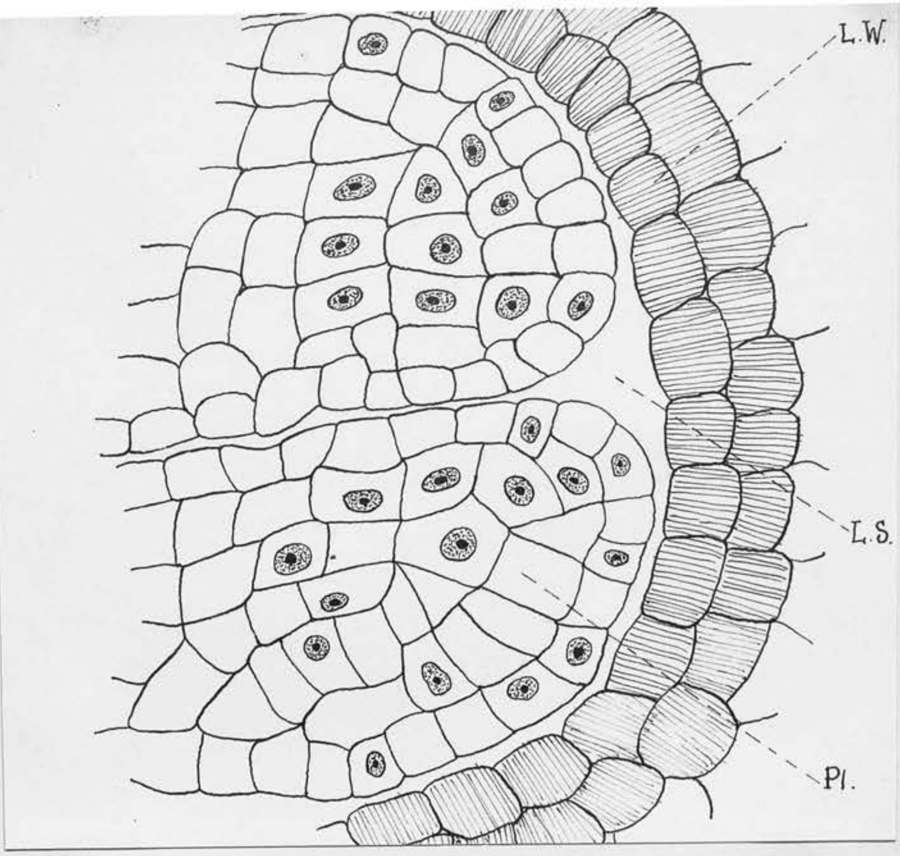

Fig. XXXVII. R. brachyanthum: T.S.Loculus, collected 8/10/31. 10 μ, x 1000.

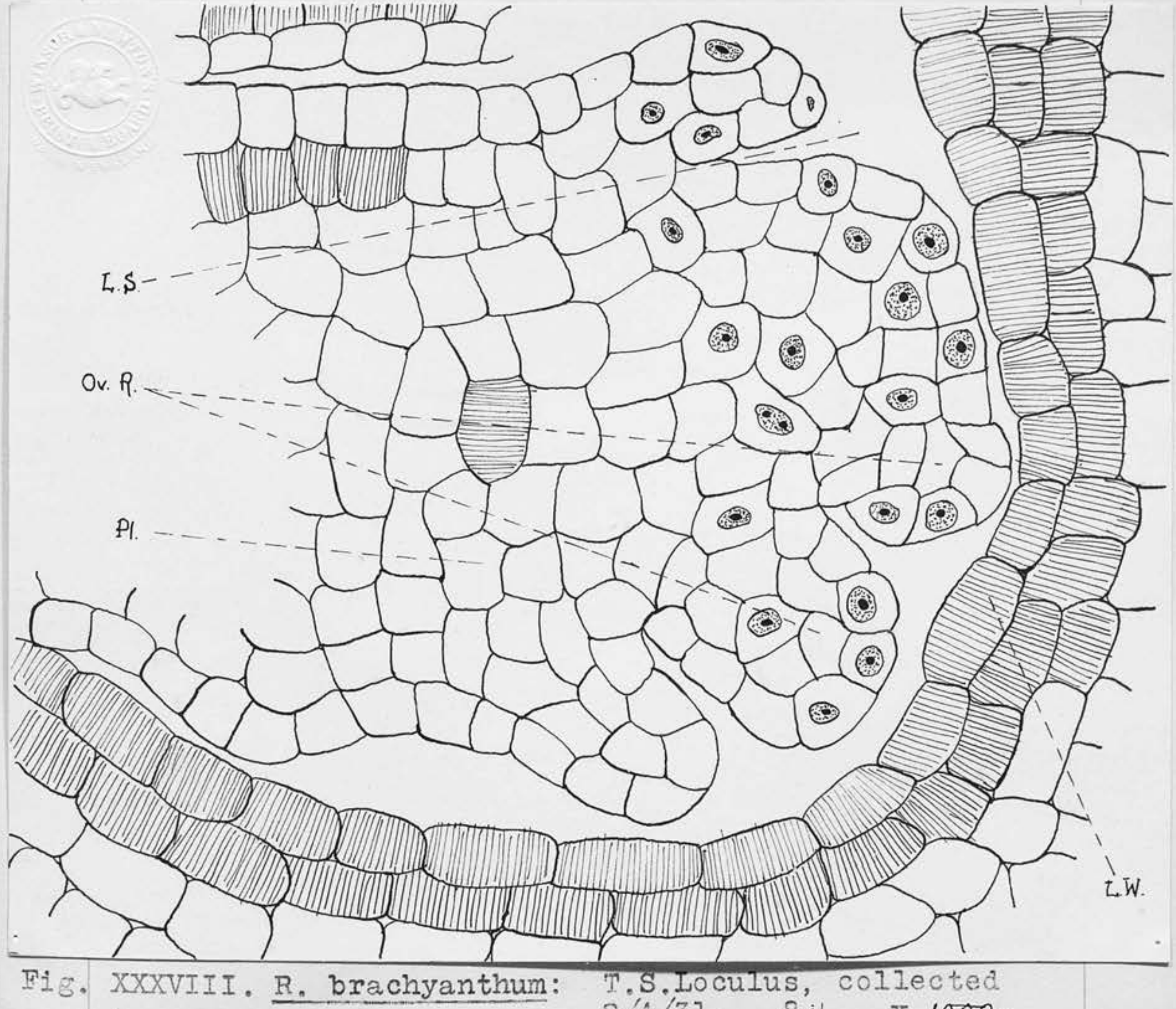

Fig. XXXVIII. R. brachyanthum: T.S.Loculus, collected 2/4/31. 8 μ, x 1000.

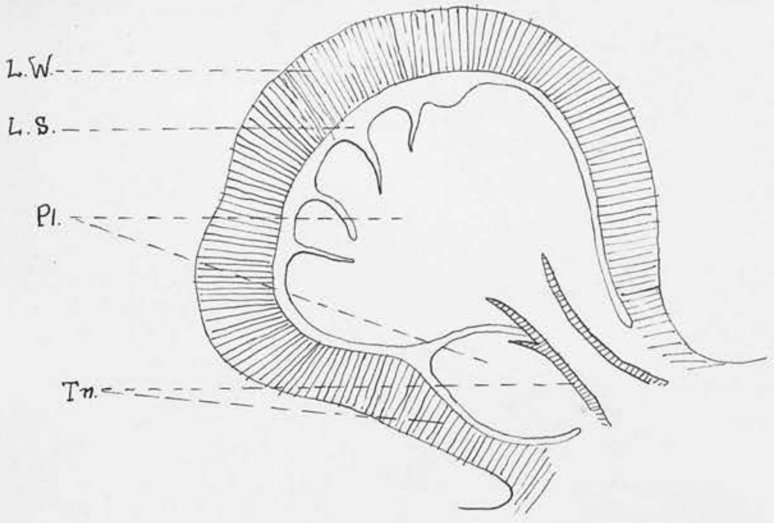

Fig. XXXIX. R. brachyanthum: T.S. Loculus (semi-diag.) showing unequal development of placentae: collected 2/4/31. 8μ, x 250.

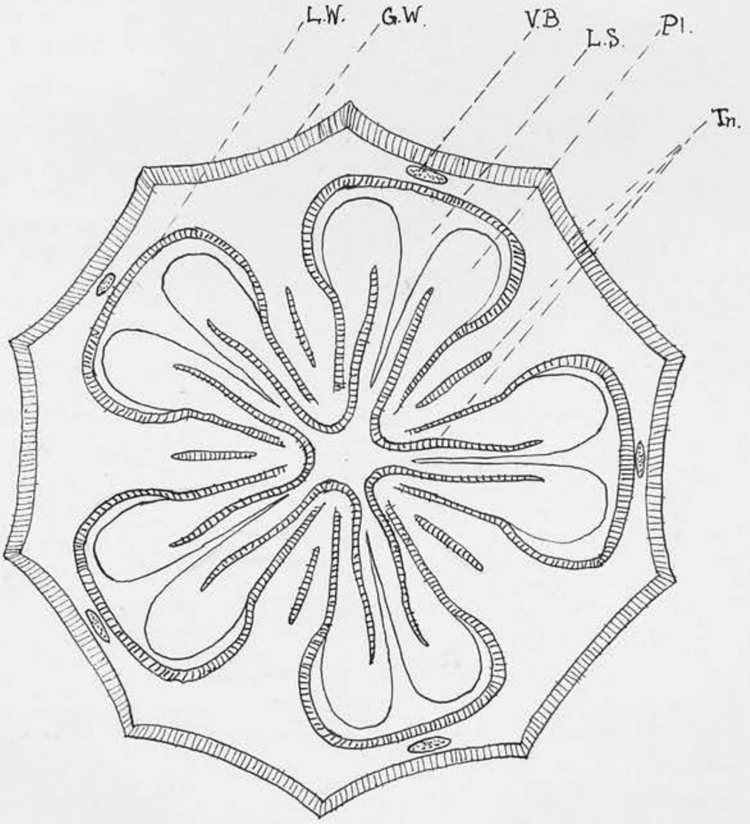

Fig. XL. R. brachyanthum: **Diagram illustrating** the distribution of Tannin and Vascular Tissue in Gynoecium collected 2/4/31.

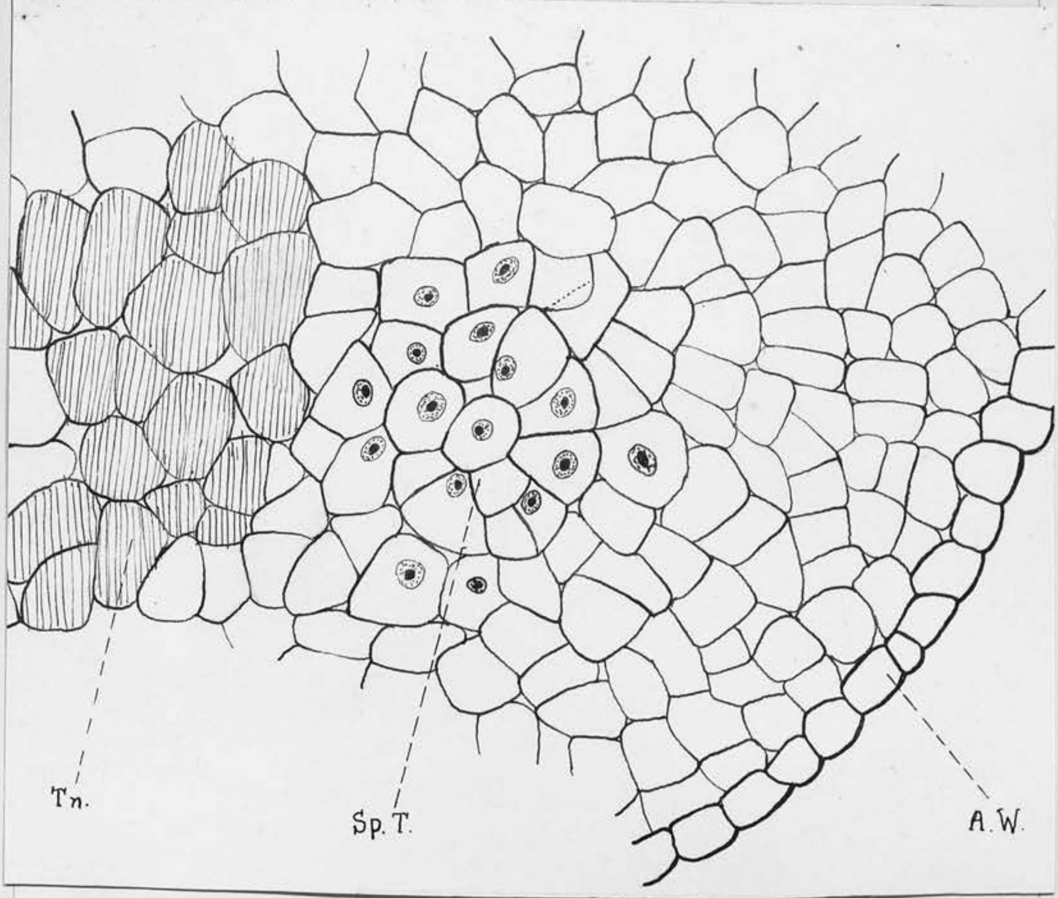

Fig. XLI. R. calophytum: T.S. Pollen Sac: collected 17/7/30. 10 μ, x 1000.

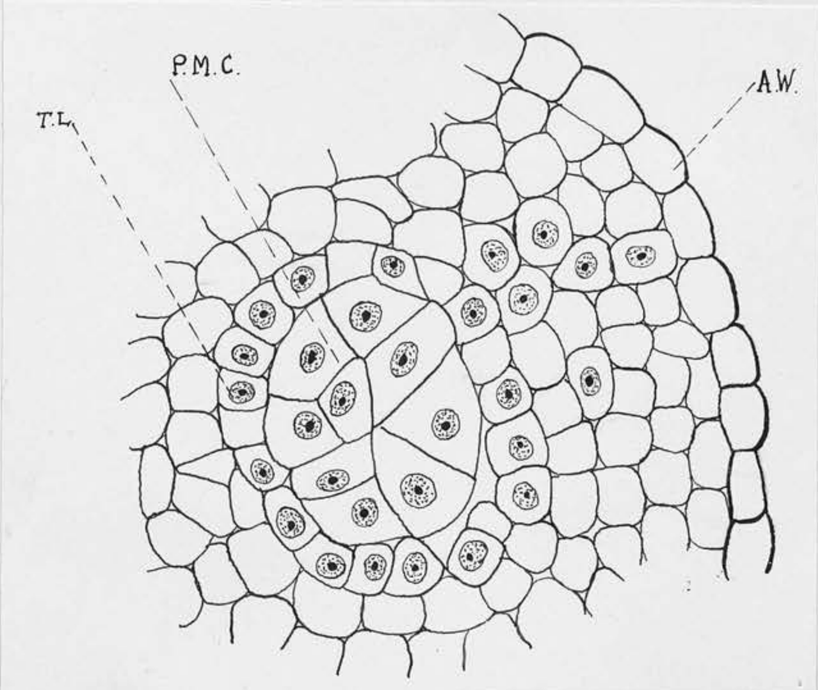

Fig. XLII. R. calophytum: T.S. Pollen Sac. collected 21/7/30. 8 μ, x 1000.

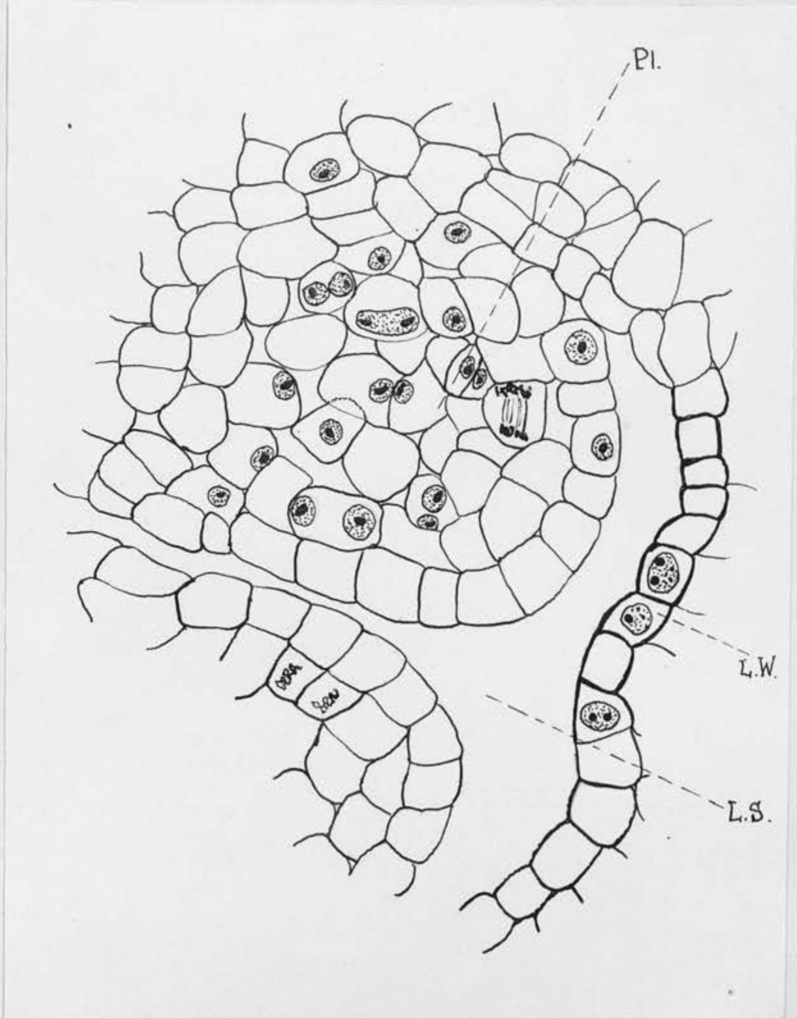

Fig. XLIII. R. calophytum: T.S. Loculus: collected 17/7/30. 6 μ, x 1000.

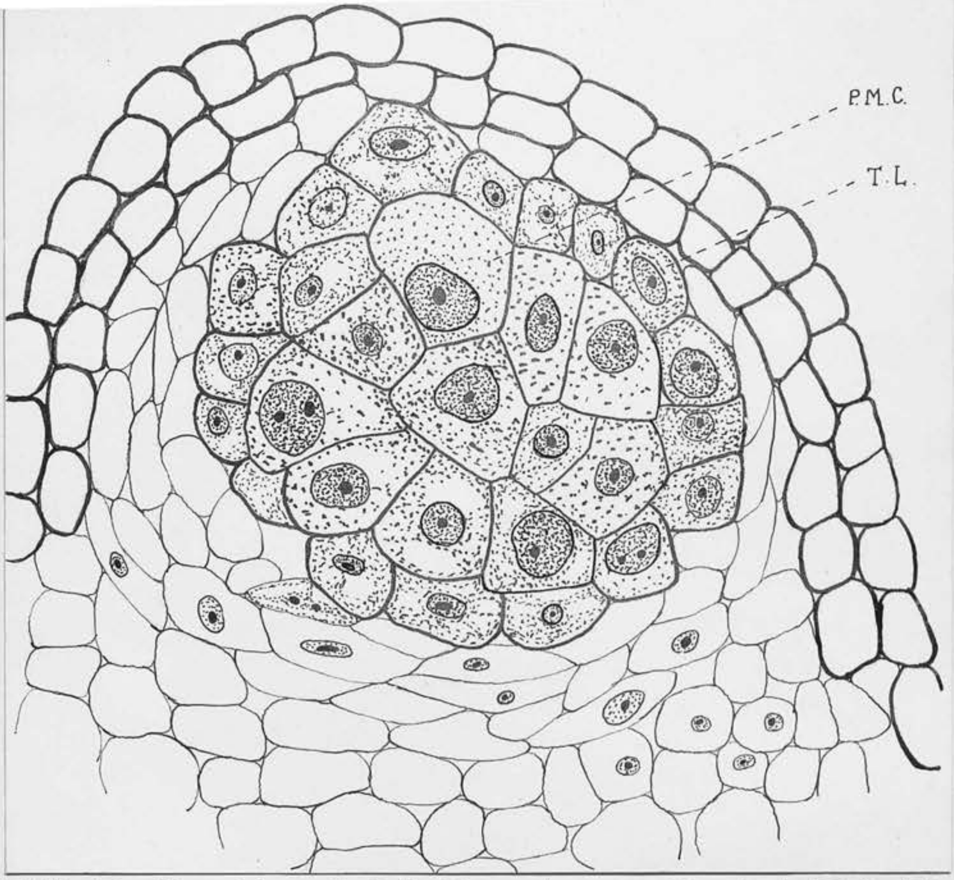

Fig. XLIV. R. Keysii: T.S.Pollen Sac: collected 16/4/31.
8 µ, x 1000.

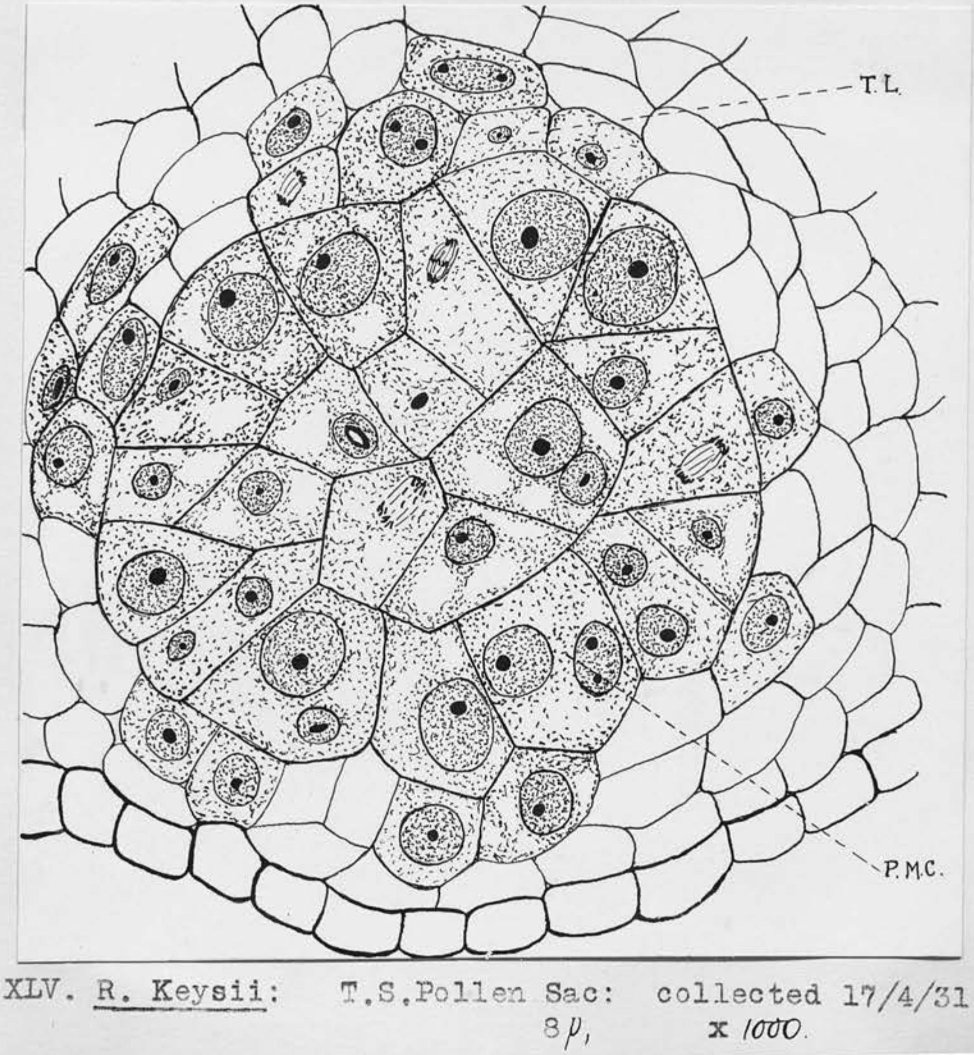

Fig. XLV. R. Keysii: T.S.Pollen Sac: collected 17/4/31.
8 µ, x 1000.

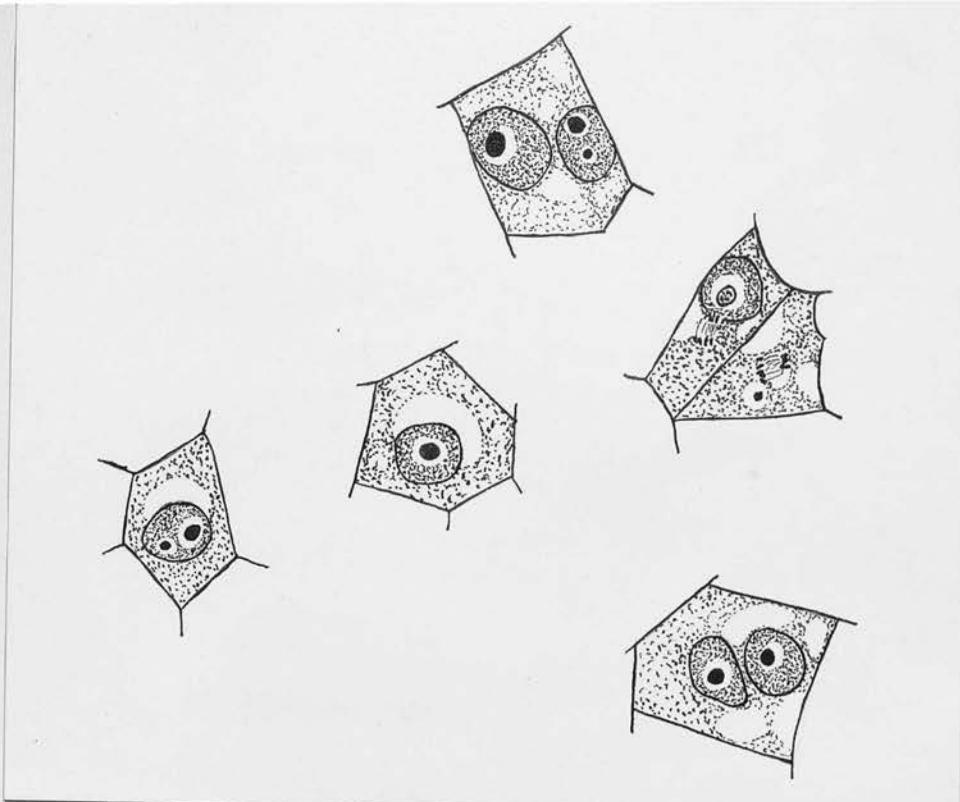

Fig. XLVI. R. Keysii: Pollen Mother Cells: collected 17/4/31. 8 μ, x 1000.

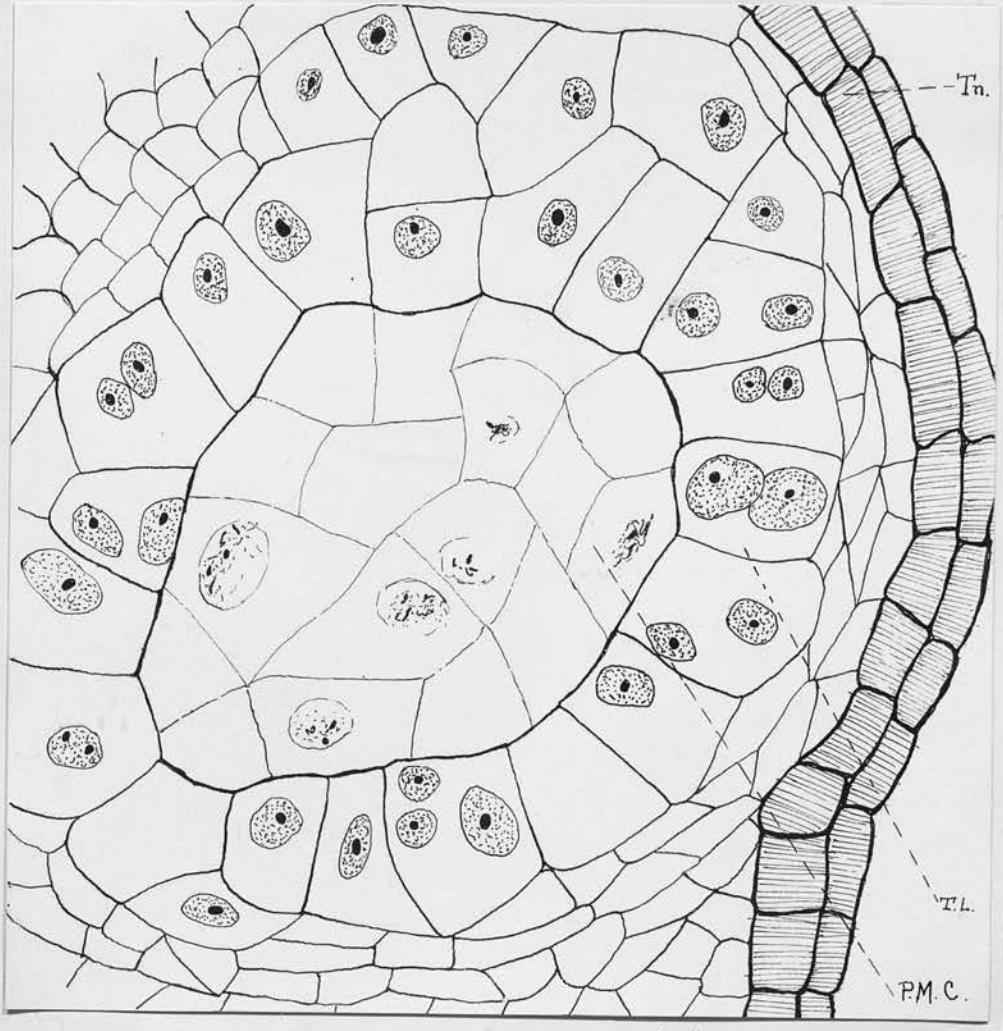

Fig. XLVII. T.S. Pollen Sac: Collected 21/4/31. 8 μ, x 1000.

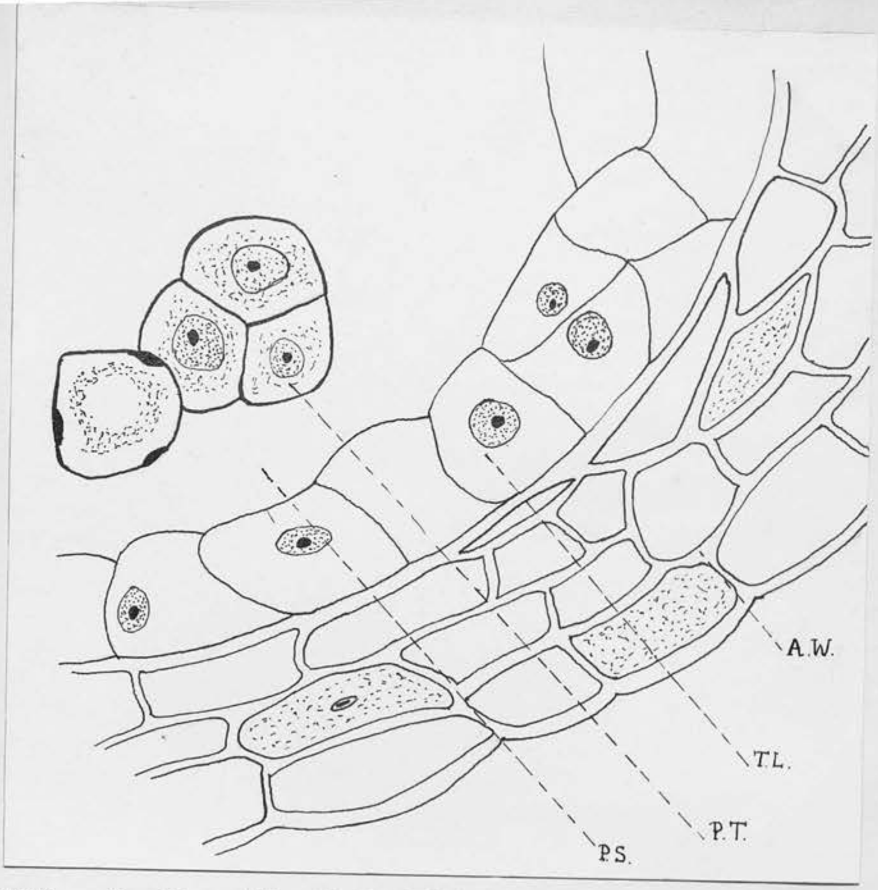

Fig. XLVIII. R. Keysii: T.S. Pollen Sac: collected 23/4/31. 8 µ, x 1000.

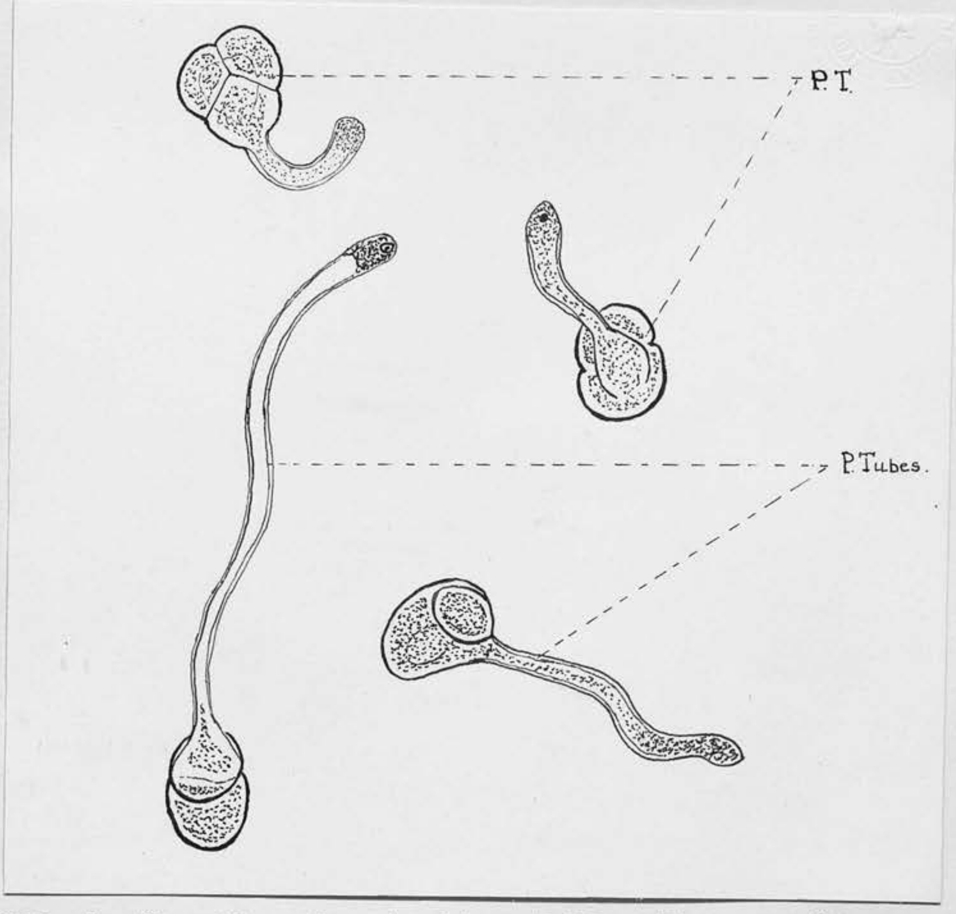

Fig. XLIX. R. Keysii: Germinating Pollen Tetrads (fresh material): Collected 16/7/31. x 250.

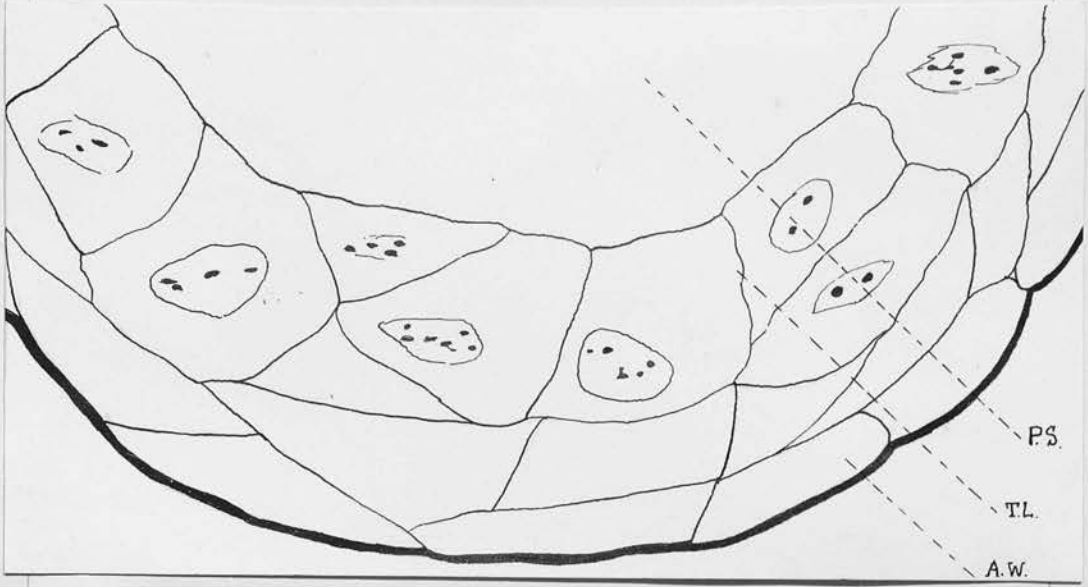

Fig. L. R. Keysii: T.S. Pollen Sac from bud from abnormal inflorescence: collected 23/4/31.
8 μ, x 1000.

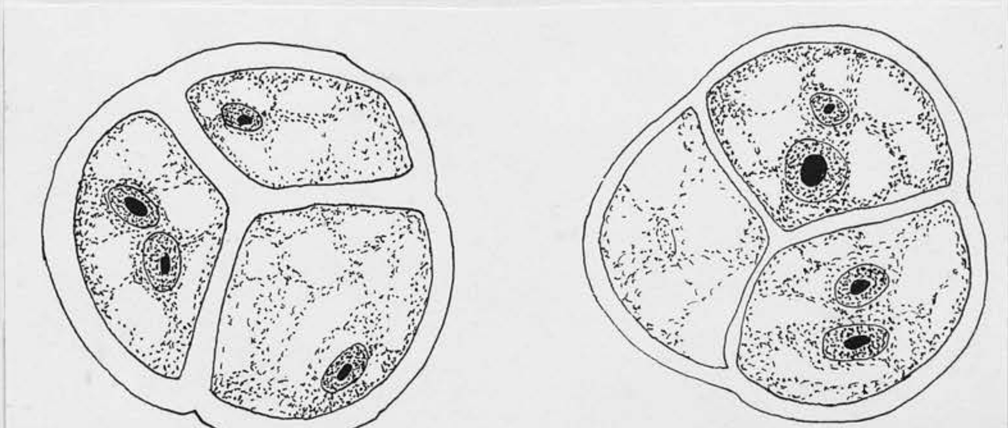

Fig. LI. R. Keysii: Pollen Tetrads from bud from abnormal inflorescence: collected 6/5/31.
8 μ, x 1000.

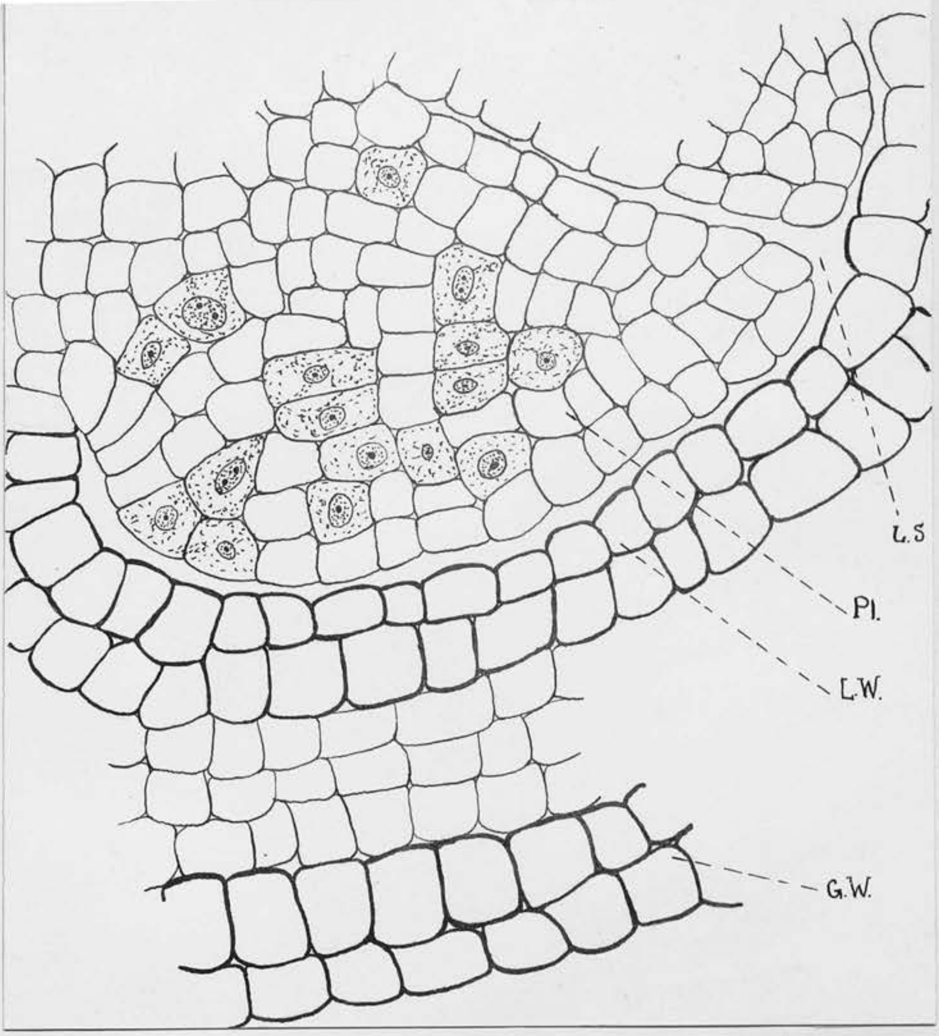

Fig. LII. R. Keysii: T.S. Loculus: collected 16/4/31.
8μ, x 1000.

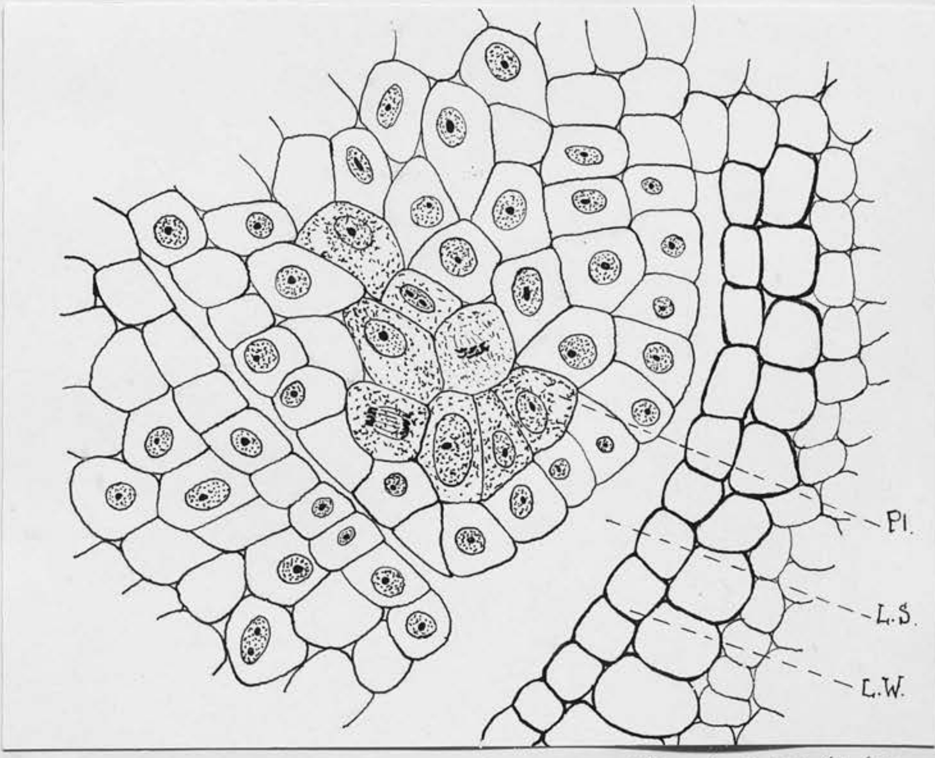

Fig. LIII. R. Keysii. T.S. Loculus: collected 17/4/31.
8μ, x 1000.

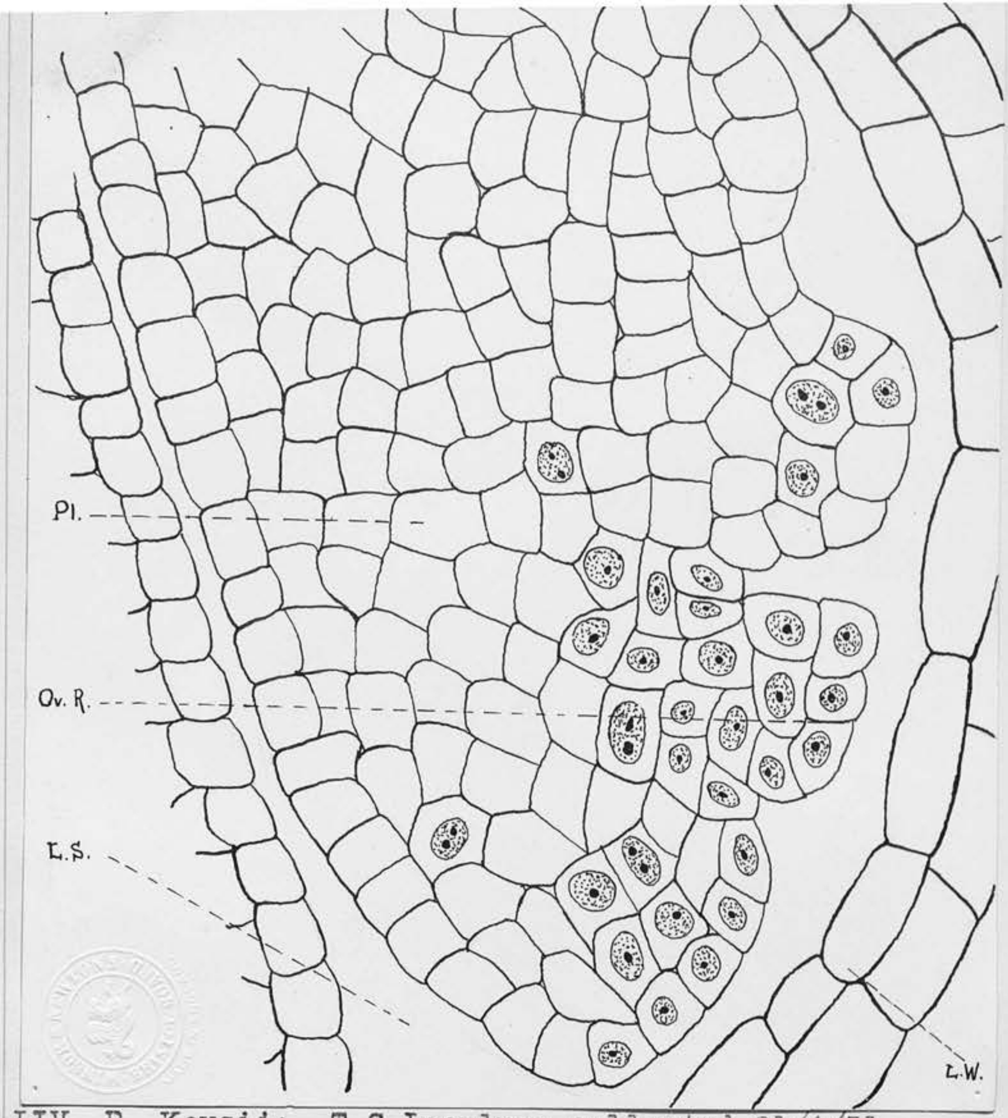

Fig. LIV. R. Keysii: T.S.Loculus: collected 21/4/31.
8 μ, x 1000.

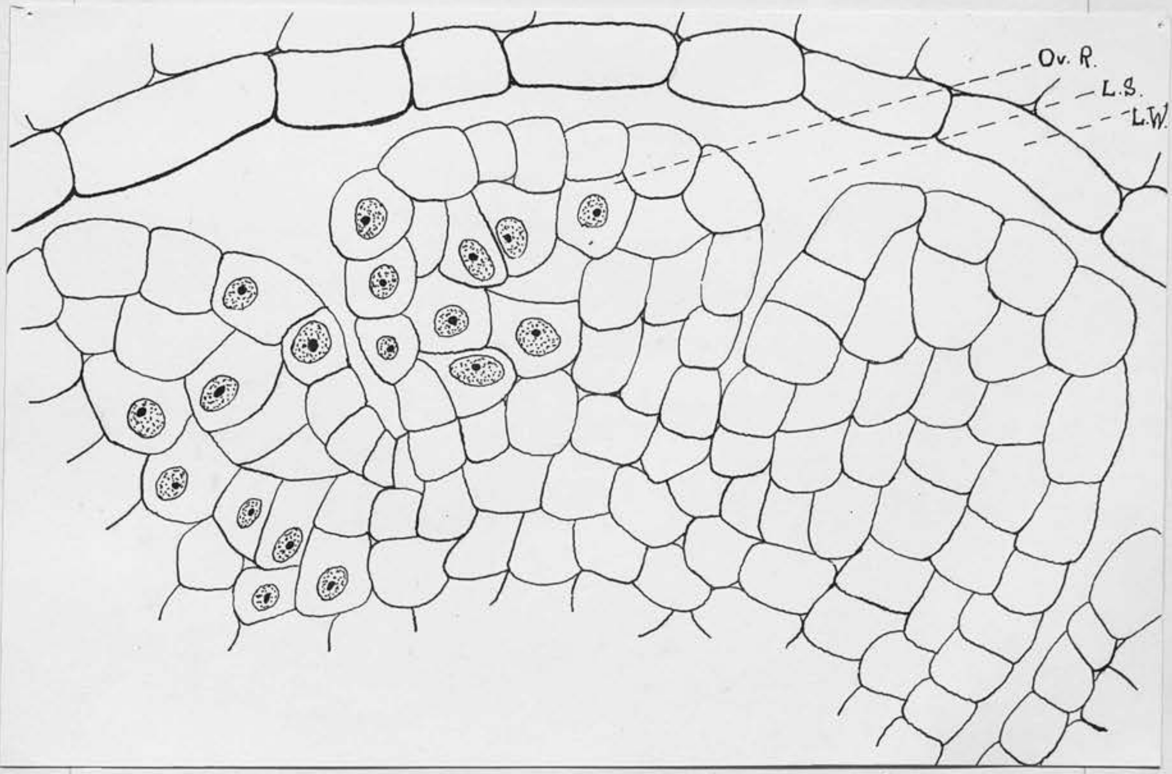

Fig. LV. R. Keysii T.S. Loculus: collected 27/5/31.
8 μ, x 1000.

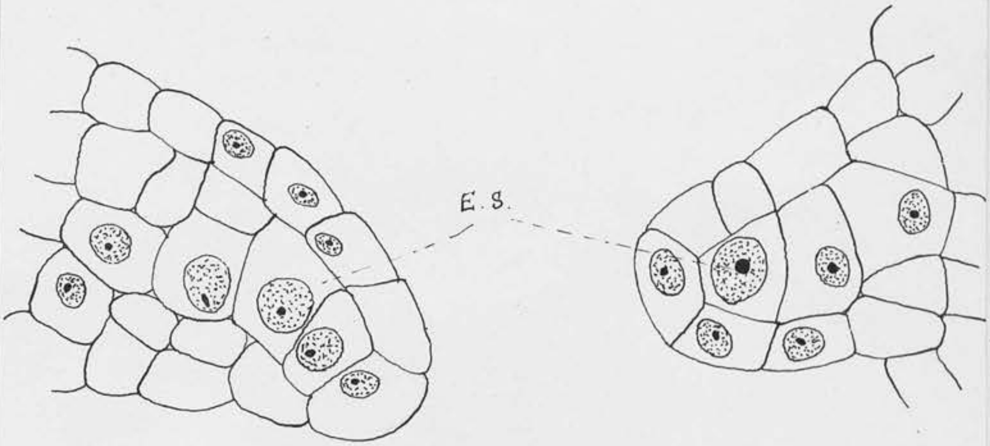

Fig. LVI. R. Keysii: L.S.Ovules: collected 10/6/31.
8µ, x 1000.

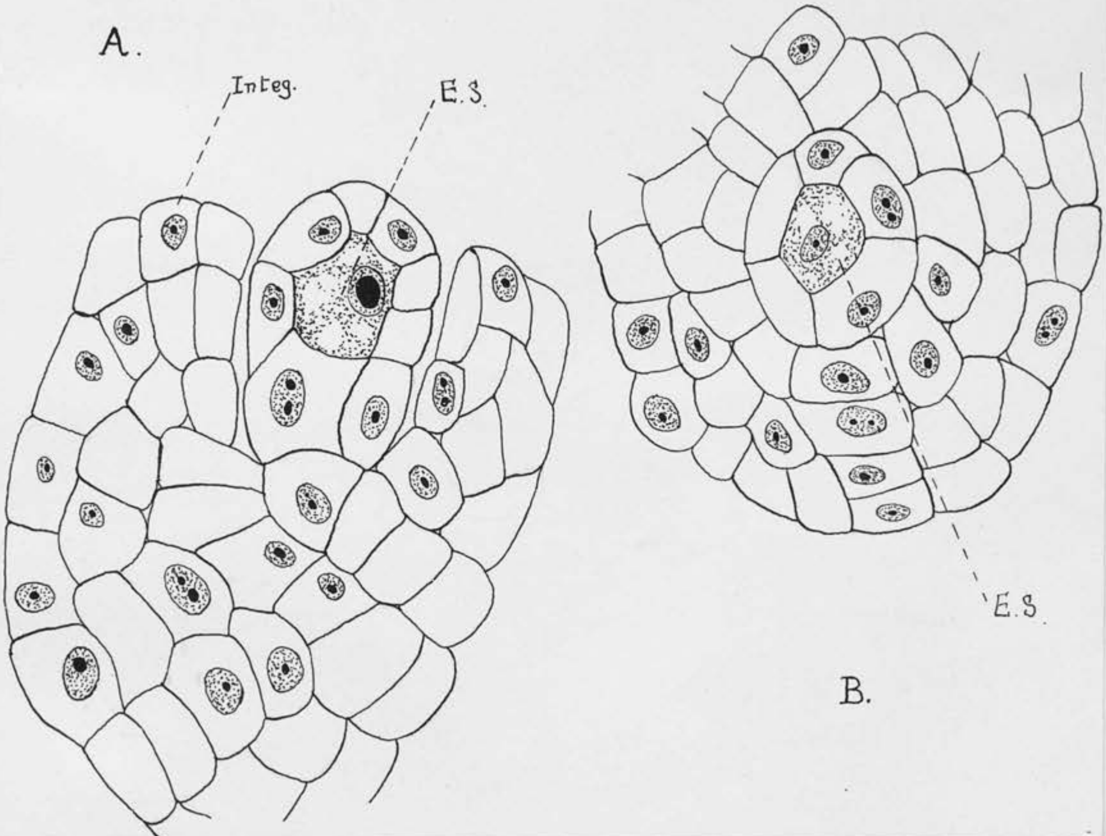

Fig. LVII.R.Keysii: (A) L.S., and (B) T.S.Ovules from bud from abnormal inflorescence collected 23/4/31. 8µ, x 1000.

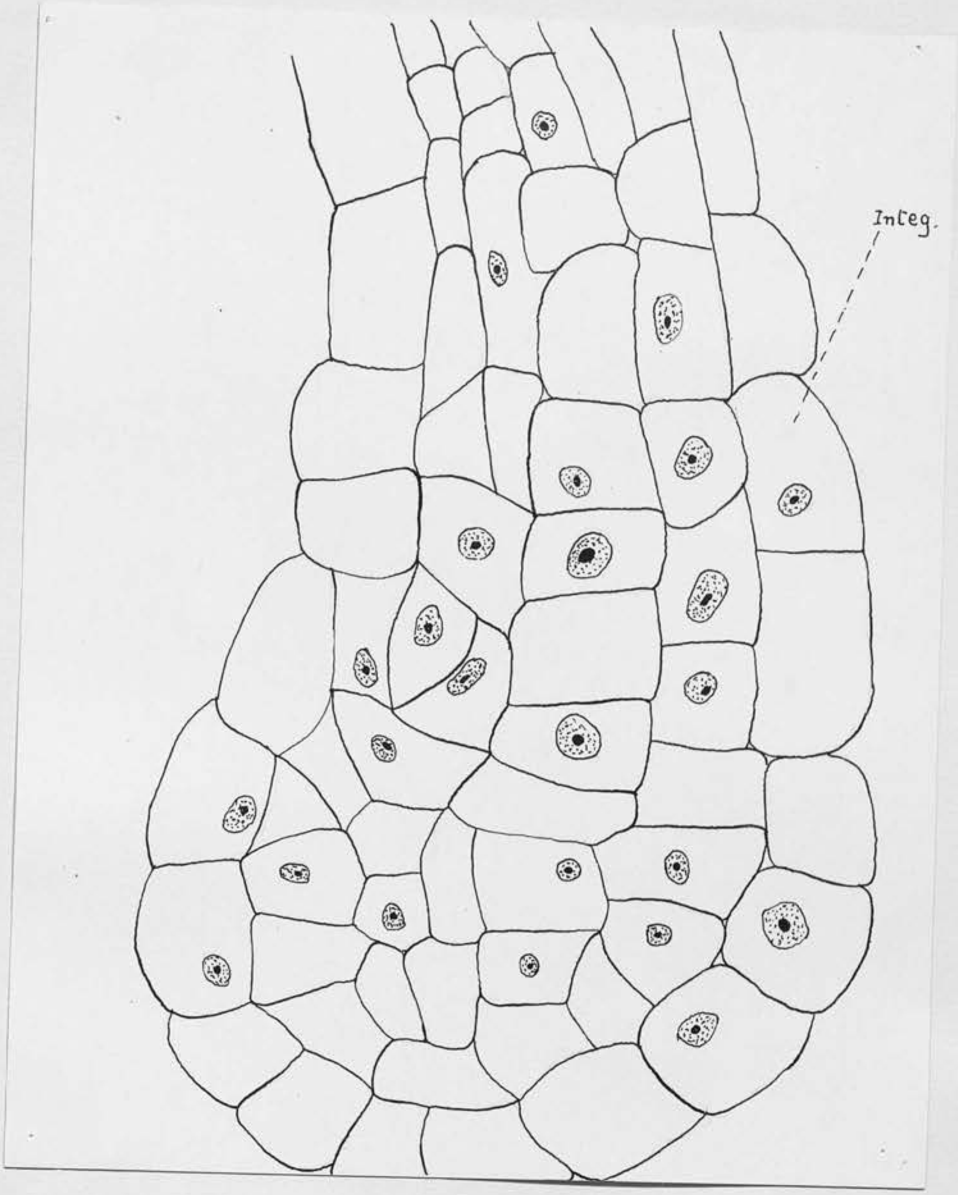

Fig. LVIII. R. Keysii: L. S. Ovule from bud from abnormal inflorescence collected 6/5/31. 8μ, x 1000.

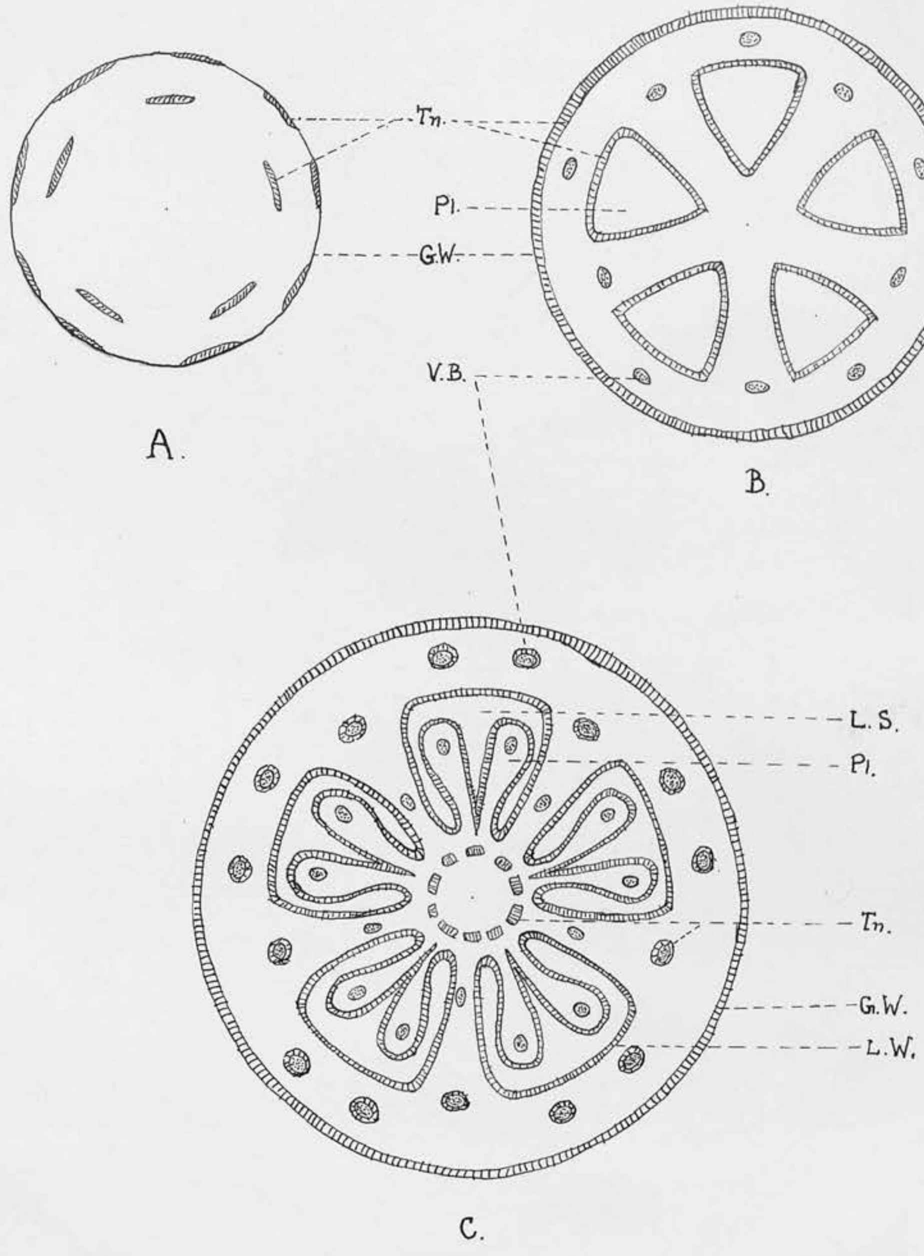

Fig. LIX. R. Keysii Diagrams illustrating the distribution of Tannin and Vascular Tissue in gynoecia of different ages.

A. gynoecium collected 2/4/31.
B. " " 16/4/31.
C. " from mature open flower.