STUDIES IN THE PROTEACEAE.

V. Vascular Anatomy of the Flower of *Grevillea robusta* Cunn.*

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Introduction.

In the Proteaceae, the vascular anatomy of the flower has so far been investigated only in *Macadamia ternifolia* F. Muell. (Kausik, 1938, 1940; Hartung and Storey, 1939). The present paper deals with another member, probably the best known of the Proteaceae, *Grevillea robusta* Cunn. Some highly interesting features have been found in this form, and it is believed that future investigations of this nature on other genera may not only bring to light many more significant facts of floral anatomy, but might also help in solving the vexed question of the affinities of the family.

Material and Methods.

The material for study, young flower buds, was fixed in Bouin's fluid and subsequently passed through the processes of dehydration and infiltration, using chloroform in place of xylol. Sections were cut at a thickness of 14 μ in both longitudinal and transverse planes, and in the latter case a complete series was secured to study the course of the vascular bundles at different levels in the floral organs. The sections were stained in either an alcoholic solution of safranin or in gentian violet; the results were satisfactory in both cases.

Observations.

The flower of *Grevillea robusta* Cunn. is borne on a well-developed pedicel and has a single whorl of four perianth leaves. The four stamens are adnate to and opposite the perianth segments. The centre of the flower is occupied by the single carpel, which is borne on a short gynophore-like stalk, and partially investing this at the base is a prominent horse-shoe shaped disc which secretes nectar (Fig. 5 shows the disc in t.s.).

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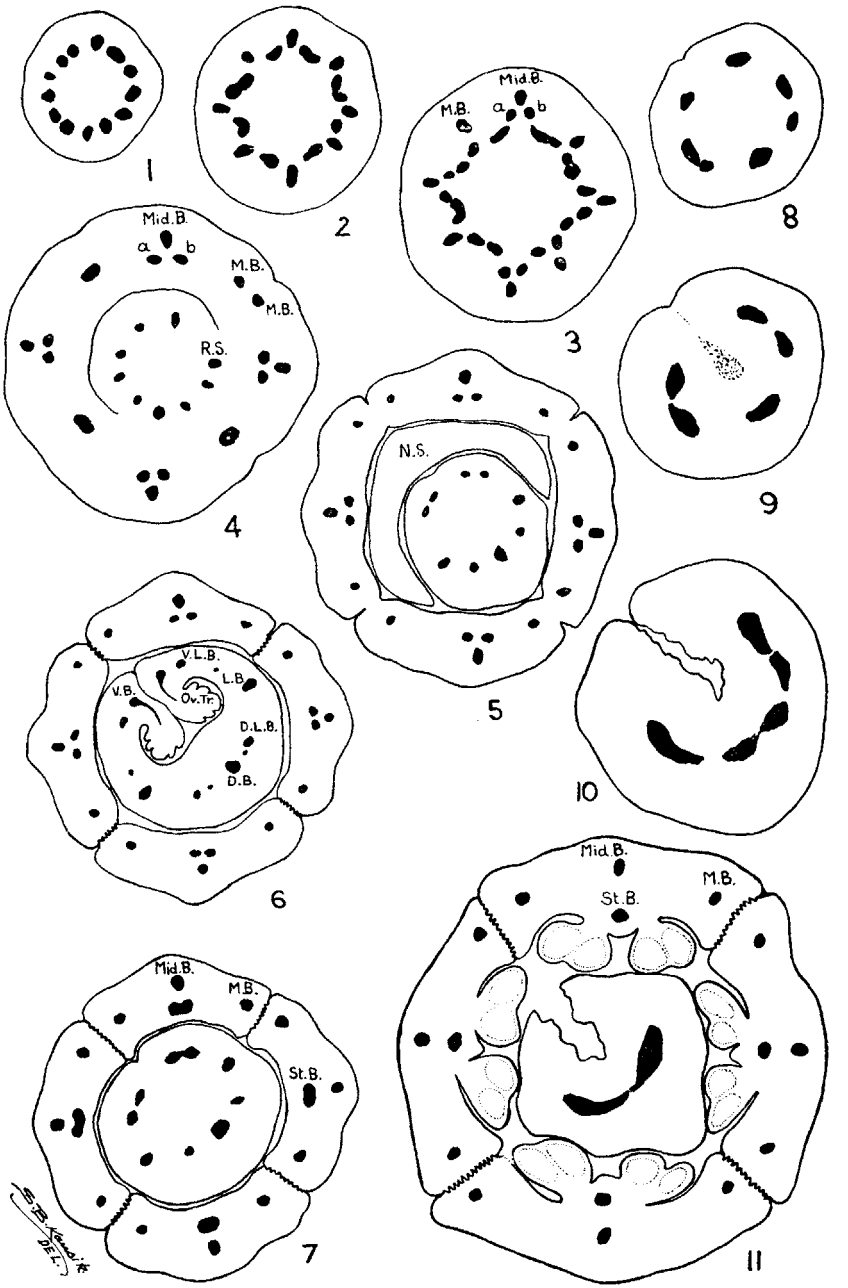
The vascular cylinder in the pedicel of the flower appears in the form of a ring in transverse sections and consists of a number of distinct bundles (Fig. 1). This ring gives rise to the first set of vascular traces to the outer floral organs at the base of the flower. There are eight traces in this set (Fig. 2); of these, four are found opposite the four perianth segments and form their midrib bundles (Fig. 3), while the other four lie on alternating radii and later divide to form the marginal bundles for the adjacent margins of the perianth segments (Fig. 4). Gaps are formed in the stele by the departure of these eight traces, and four of these alternating gaps are common to both the midrib bundles of the perianth and the apposing stamen traces which are formed at approximately the same level (Fig. 3: *a* and *b*). Further, it is interesting to note that the stamen traces are distinctly double in origin consisting of two bundles forming a pair (Fig. 17 and Pl. I, Fig. 1). Four pairs of such bundles corresponding to the four stamens are next seen at successively higher levels in the floral receptacle taking a more or less vertical course and lying internal to the four midrib bundles of the perianth (Figs. 4–6). Alternating with these are seen the traces which divide higher up in the receptacle to form the marginal bundles belonging to adjacent margins of the perianth.

The vascular bundles of the outer floral organs next enter the bases of the perianth segments when the latter become free from the receptacle of the flower (Fig. 5). The paired arrangement of the bundles in the stamen traces is clearly seen even here and persists so for a considerable distance upwards (Fig. 6 and Pl. I, Fig. 2). Above the level of the ovules, as seen in transverse sections of the flower, the two bundles of each pair gradually approach each other and finally fuse together to form a single bundle (Figs. 7 and 11 and Pl. I, Fig. 3). This resultant stamen bundle is almost twice as large as the midrib bundle of the perianth and now proceeds directly to the base of the anther. It leaves the perianth segment at the level at which the stamen is no longer adnate.

Brough (1933) in his paper on the life-history of *Grevillea robusta* gives outline figures of a series of transverse sections of the flower at various levels. In two of these figures (cf. Fig. 20 and 21) the vascular bundles of the perianth segments are shown, but the stamen bundles in the perianth segments are not marked. Another figure (cf. Fig. 14), however, representing a transverse section of the flower at the level of the ovules shows a single bundle internal to the midrib bundle of the perianth segment. This is larger than the midrib bundle and is evidently the fused stamen bundle, but he does not make any comment on its large size or origin.

In view of the unusual nature of the stamen traces, the histological details of the bundles at various levels were closely studied. At the level of separation from the receptacular stele, the paired stamen bundles are disposed more or less tangentially. In each pair, the two xylem groups are towards the inside facing each other, while the phloem groups form two large arc-like tissues on the outside. The inner ends of these arc-like phloem groups disappear higher



Grevillea robusta Cunn.

Text-figs. 1–11. Outline diagrams of transverse sections of the flower from the pedicel upwards to show the vascular bundles in the different floral organs; in Figs. 8–10 only the style and stigma are shown in outline. Fig. 1. ×51; Figs. 2, 4, 5–7 and 9. ×42; Fig. 3. ×40; Fig. 8. ×45; Fig. 10. ×38; and Fig. 11. ×25.

R.S.—Receptacular Stele; Mid.B.—Midrib Bundle of perianth segment; M.B.—Marginal Bundle of perianth segment; *a* and *b*—The two bundles forming the paired vascular trace to a single stamen; St.B.—Stamen Bundle formed by fusion of *a* and *b*; D.B.—Dorsal Bundle in carpel; D.L.B.—Dorso-lateral Bundle in carpel; L.B.—Lateral Bundle in carpel; V.B.—Ventral Bundle in carpel; V.L.B.—Ventro-lateral Bundle in carpel; Ov. Tr.—Ovule Trace; and N.S.—Nectar-secreting disc.

up at the base of the perianth and the bundles become typically collateral in nature. This condition is seen without any further change during the passage of the stamen bundles in the perianth segments. Later the two bundles of the pair approach each other closely as the level of fusion is reached and finally give rise to a single large bundle, also collateral in nature. This large bundle next proceeds directly to the base of the anther. The structure and manner of fusion of the bundles are shown in Figs. 17–19 and in the photomicrographs.

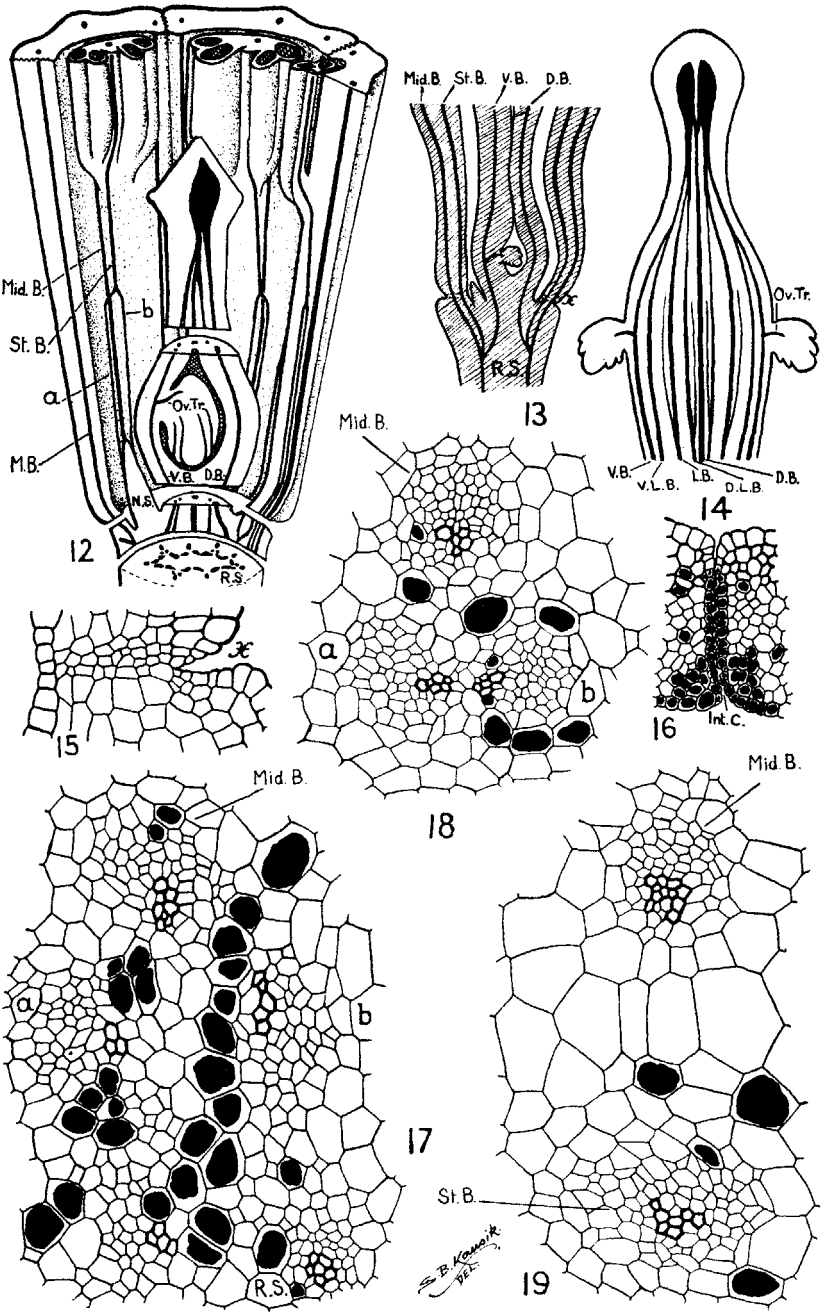
After the departure of the traces to the perianth segments and the stamens, the remaining vascular bundles again form a ring which 'shrinks' to the centre of the receptacle. At first this ring is composed of a variable number of bundles of different sizes, but at a slightly higher level only nine bundles become rather prominent (Fig. 4). These form the main vascular supply of the carpel, while the others fade away in the tissue of the floral axis.

The nine bundles destined for the carpel enter the gynophore and are disposed almost in the form of a ring, in which, however, a definite orientation of the bundles with reference to the carpel can be made out (Figs. 4 and 5). It is, therefore, probable that the gynophore-like stalk is really carpellary in nature; its somewhat radial organization seems to be only an apparent condition, for it may be assumed with some justification that the apex of the floral shoot actually terminates at the base of the gynophore at the level at which the residual vascular tissue, namely that remaining after the formation of the carpellary bundles, is discarded into the tissue of the floral axis. Further, it is probable that the solitary carpel is only pseudoterminal in its position like that of the Leguminosae (Eames, 1931).

Of these nine carpellary bundles, one forms the large dorsal bundle, two slightly smaller dorso-lateral bundles disposed on either side, two lateral bundles, two ventral bundles, and lastly two ventro-lateral bundles lying in a submarginal position and parallel to the ventral bundles (Fig. 5). At a slightly higher level, especially where the ovules are borne, the two dorso-lateral bundles and the two lateral bundles give rise to additional bundles by dividing once (Figs. 6 and 14), raising the total number to as much as thirteen. The traces for the two ovules are formed by the two ventral bundles of the carpel (Figs. 6 and 12–14).

The formation of such a large number of bundles in the carpel is a striking feature. This appears to be due to the carpel's extra demand for nutrition during its increase in size, especially at the level of the ovules. Brough (1933) also remarks that an 'increase in size of carpel results in a marked increase in the number of vascular tracts until . . . . as many as 13 bundles may be observed'.

Above the level of the ovules the extra bundles fade away in the tissue of the carpel so that the original nine bundles are again restored at the base of the style (Figs. 7, 14). These bundles continue their upward courses in the style, but as the stigmatic region is approached the ventral bundles fuse with the ventro-laterals on the two corresponding sides of the carpel (Figs. 7, 8).



Grevillea robusta Cunn.

Text-figs. 12–19. Fig. 12. Three dimensional diagram of flower showing the different sets of vascular bundles. Fig. 13. Diagrammatic longitudinal section of the lower part of the flower to show the relationships of the vascular bundles of the different floral organs to the receptacular stele. Fig. 14. Diagram of carpel as if opened out and placed in one plane to show the vascular bundles of the carpel and the formation of anastomoses in the regions of the style and stigma. Fig. 15. Base of the perianth segment showing the absciss layer marked *x*. ×100. Fig. 16. Adjacent margins of two perianth segments showing the interlocking arrangement. ×100. Figs. 17–19. Midrib portions of the perianth segment at various levels to show the midrib bundle and the associated stamen trace. ×225. Fig. 17. At the level of separation from the receptacular stele; the double stamen trace is marked by *a* and *b* in this and also in the next figure. Fig. 18. At a higher level in the perianth segment. Fig. 19. At a still higher level showing the fused condition of the stamen trace. Figs. 12–14 are diagrammatic and are not drawn to a measured scale. Int.C.—Interlocking cells of the margin of perianth; *x*—Absciss layer at the base of perianth; the rest as in Figs. 1–11.

There are thus seen in transverse sections of the style at this region only five large bundles, of which the dorsal bundle is still recognizable as before, while the other four form two pairs on either side (Fig. 9). Gradually the bundles of each pair also approach each other and finally fuse together (Figs. 9, 10). At this time the dorsal bundle splits into two equal parts, each of which next fuses with the resultant bundles on either side of the carpel (Figs. 10, 11). Transverse sections of the stigma, therefore, show only two large vascular masses, which eventually disappear at the tip (Figs. 12, 14). These events are diagrammatically depicted in Fig. 14.

Transverse sections of the horse-shoe shaped nectar-secreting disc which is found at the base of the ovary failed to give any indication of a vascular supply. Brough (1933) also states that it consists entirely of thin-walled parenchyma without any differentiation. The absence of vascular tissues in the disc is noteworthy, for, the same organ shows some feebly developed bundles in an allied member of this family, *Macadamia ternifolia* (Hartung and Storey, 1939; Kausik, 1940). The significance of this will be dealt with in the next section of this paper.

Discussion.

Judging from the nature of the vascular supply, namely the presence of three traces for each segment, the perianth in the Proteaceae is probably to be considered as corresponding to the calyx whorl, and it is likely that the next inner whorl, the corolla, has been lost during the course of evolution. Some evidence for such a surmise was available in *Macadamia ternifolia* (Kausik, 1940), in which the nectar-secreting disc shows vascular traces derived from the receptacular stele and is, therefore, supposed to represent the remnants of a corolla. In the case of the Thymelaeaceae, a family regarded by some (e.g. Hutchinson, 1926) to be probably related to the Proteaceae, Joshi (1936) observes that the disc-scale in *Stellera chamaejasme* Linn. probably constitutes a much reduced part of the corolla as it contains vascular bundles. In the case of *Grevillea robusta*, however, the disc does not show any bundles. This led Brough (1933) to remark that while the suggestion occurred to him that it might 'constitute a reduced and modified inner whorl of floral leaves, or in other words represent the vestiges of an ancestral normal corolla.....', 'there is no indication, whatsoever, of any associated vascular tissue, and consequently no ground for interpreting the scale as the morphological equivalent of a reduced perianth.' Nevertheless, from what is now known from a study of a closely related form, namely *Macadamia ternifolia* (Hartung and Storey, 1939; Kausik, 1940), the opinion expressed by Brough requires revision. Further, additional support for the petaloid nature of the disc seems also to be available from the fact that it may also be represented in this family, as in *Banksia*, by four scale-like structures alternating with and internal to the four perianth segments. In this connection, it is interesting to recall the following remarks by Wilson and Just (1939)—'It is undoubtedly true that in some cases

at least, what may appear to be rudimentary organs have persisted with no corresponding vascular supply; such structures may, however, be really organs which can only be interpreted by a comparative study of other and closely related forms.' Again, Arber (1933) also observes that organs may often persist even though the vascular traces supplying them have disappeared completely. The disc in *Grevillea robusta* is probably such a vestigeal organ with no vascular supplies to indicate its true morphological nature, but which can still be interpreted correctly by comparison with a related form like *Macadamia ternifolia*. It is, however, true that vascular bundles may sometimes appear on account of purely physiological demands. But it is surprising, and also difficult to explain, why this physiological necessity should arise only in *Macadamia ternifolia* and not in *Grevillea robusta*, although the disc is equally prominent.

Another noteworthy feature in the floral anatomy of *Grevillea robusta* is the nature of the stamen traces which consist of paired bundles. Generally, the stamen in angiosperms possesses a single vascular bundle, and this condition is attributed to the much attenuated nature of the organ (Eames, 1931). However, stamens with three bundles (Magnoliaceae, Musaceae and Lauraceae) are sometimes met with. Eames (1931) remarks that 'since from other evidence, these appear to be fairly primitive groups, it seems highly probable that the single trace condition is one of reduction from three.' In the case of the avocado, *Persea americana* Mill., belonging to the Lauraceae, Reece (1939) has recently shown that while the stamens of the first two whorls have three bundles at the base with the two laterals disappearing above, there are five bundles at the base of each stamen in the third whorl. He remarks that the condition in the avocado stamen probably indicates the retention of a primitive character.

The condition in *Grevillea robusta* with a distinct pair of bundles for each stamen is an extremely rare one. Joshi and Rao (1934) have noted that each stamen trace in *Digera arvensis* Forsk. consists at first of a single bundle, but that it very soon 'divides into two which remain as such throughout the length of the staminal tube and fuse again at the level of the separation of the stamens from the staminal tube.' They state that the explanation for this behaviour seems to be the same as that given by Sahni (1961) in the case of the tubers of *Nephrolepis* and *Equisetum*, namely that an 'increase in the diameter of an organ tends to cause a corresponding dilatation of its vascular system.' They further remark that the vascular supply of the stamens in *Digera* furnishes an illustration of the hypothesis of 'Size and Form' (Bower, 1930).

Arber (1932) has described two-bundled stamen supplies in the genus *Hypecoum* of the Fumariaceae. She observes that this character is merely due to a special type of bundle branching associated with the superposition of stamen and petal and remarks: 'Whether the bundle pairs retain their

independence or fuse appears to depend at least in part—upon the relative width of the filament base.' In the case of *Grevillea robusta* the paired condition remains as such for a considerable distance during the passage of the stamen trace in the perianth segment and the fusion of the two bundles occurs only at a higher level without there being any appreciable reduction in the size of the organ that carries the trace (i.e., the perianth segment). The fused bundle enters the base of the anther only at a much higher level than this, and at lower levels both the double and the fused conditions are seen. There is, therefore, no ground to assume that fusion is due to any want of space for the independence of the bundles between the level at which fusion takes place and that at which the fused bundle actually enters the base of the anther. For these reasons, the explanation offered by Joshi and Rao (1934) for the doubling of the bundles in *Digera arvensis* is also unsatisfactory in the present case; for, even if the double nature of the stamen trace is regarded to represent an amplification of the stamen vascular system, the question of size as a factor determining such an amplification does not seem to arise at all here.

Saunders found branching stamen traces in the Cistaceae (1936 *a*) and in the Hypericaceae and Lythraceae (1936 *b*), and Wilson (1937) reports having seen a similar condition in the Tiliaceae and Anonaceae. Wilson (1937) has also made a careful study of the stamen traces in the Parietales and the Malvales, in which the stamens are in fascicles, and has put forth the view that the modern angiosperm stamen is derived from a dichotomous branch system. On this basis, the prevailing type of anther with its four sporangia is regarded as consisting of a pair of bisporangiate synangia, and this type is derived through reduction from a dichotomously dividing branch system. Wilson remarks: 'If then these two orders be considered as conservative groups, containing forms which have retained certain of the ancestral features of the progenitors of all Angiosperms, the stamen throughout the flowering plants is phylogenetically of the same nature.....'

The primitive branching tendency of the stamen trace is also met with in a few other cases, as *Gloriosa superba* (Joshi, 1939) and *Persea americana* (Reece, 1939). The paired condition in *Grevillea robusta* suggests a similar tendency and it is noteworthy that the paired arrangement is seen not only at higher levels, but also at the point of separation of the trace from the receptacular stele. In the forms studied by Wilson the stamen fascicle traces are single at the point of origin, except perhaps in *Symphonia globulifera* L., in which the combined petal-stamen fascicle supplies break up into an outer 'horse-shoe shaped mass of tissue which proceeds directly to the base of a petal, together with two inner bundles which form the supply to a single fascicle.'

After considering the different possibilities, it seems that the hypothesis put forth by Wilson that the stamen is derived from a dichotomously dividing branch system offers the most plausible explanation for the unusual nature of the stamen supply in *Grevillea robusta*.

Summary.

The paper deals with the floral anatomy of *Grevillea robusta* Cunn.

The receptacular stele appears in the form of a ring in transverse sections. It first gives rise to a set of eight traces for the perianth. Four of these alternating traces later divide once to form the marginal bundles for the perianth segments, while the other four directly become the midrib bundles.

The stamen traces arise from the same gaps from which the midrib bundles depart. Each stamen trace is distinctly double at the point of origin and this condition continues for a considerable distance upwards. The two bundles fuse together above the level at which the ovules are seen in transverse sections.

The double nature of the stamen supply is both unusual and remarkable. It probably suggests the derivation of the stamen from a dichotomizing branch system.

No vascular tissue is seen in the nectar-secreting disc which lies at the base of the ovary, but the same organ in another member of this family, *Macadamia ternifolia*, shows the presence of feebly developed vascular bundles. It is, therefore, probable that as in *Macadamia* the disc is here also of the nature of a vestigial inner floral envelope, namely the corolla. The modern Proteaceae may thus be regarded as derived from an original dichlamydeous condition, and the simplicity of floral structure is due to reduction.

In conclusion, it is a pleasure to thank Dr. P. Maheshwari, Dacca University, for kindly reading through the manuscript and making many useful suggestions, and Professor M. A. Sampathkumaran, Head of the Department of Botany, University of Mysore, for his kindness and encouragement during this work.

Literature cited.

Arber, A., 1932. Studies in Floral Morphology IV. *New Phytol.*, *31*: 145–73.

————, 1933. Floral Morphology and its morphological interpretation. *Ibid.*, *32*: 231–42.

Bower, F. O., 1930. Size and Form in Plants, London.

Brough, P., 1933. The life-history of *Grevillea robusta* Cunn. *Proc. Linn. Soc. N.S.W.*, *58*: 33–73.

Eames, A. J., 1931. The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. *Amer. Journ. Bot.*, *71*: 147–88.

Hartung, M. E. and Storey, W. B., 1939. Development of the Fruit of *Macadamia ternifolia*. *Journ. Agri. Res.*, *59*: 397–408.

Hutchinson, J., 1926. Families of Flowering Plants, Vol. 1, London.

Joshi, A. C., 1936. Anatomy of the flowers of *Stellera chamaejasme* Linn. *Journ. Ind. Bot. Soc.*, *15*: 77–86.

————, 1939. Vascular Supply of the Stamens and Ovules of *Gloriosa Superba*. *Nature*, *143*: 437.

———— and Rao, C. V., 1934. A contribution to the Anatomy, Morphology and Cytology of the Flower of *Digera arvensis* Forsk. *Journ. Ind. Bot. Soc.*, *13*: 201–36.

Kausik, S. B., 1938. Studies in the Proteaceae II. *Proc. Ind. Acad. Sci.*, B., *8*: 45–62.

————, 1940. Vascular Anatomy of the Flower of *Macadamia ternifolia* F. Muell. (Proteaceae). *Curr. Sc.*, *9*: 22–5.

Reece, P. C., 1939. The floral anatomy of the avocado. *Amer. Journ. Bot.*, *26*: 429–33.

Sahni, B., 1916. The vascular anatomy of the tubers of *Nephrolepis*. *New Phytol.*, *15*: 72–80.

Saunders, E. R., 1936 (*a*). The vascular ground plan as a guide to floral ground plan: illustrated from Cistaceae. *New Phytol.*, *35*: 47–67.

————, 1936 (*b*). On rhythmic development and radial organization in the flower. *Journ. Linn. Soc., Bot.*, *50*: 291–322 (cited from Wilson, C. L., 1937).

Wilson, C. L., 1937. The phylogeny of the stamen. *Amer. Journ. Bot.*, *24*: 686–99.

Wilson, C. S. and Just, T., 1939. The Morphology of the Flower. *Bot. Rev.*, *5*: 97–131.

Explanation of Plate I.

Grevillea robusta Cunn.

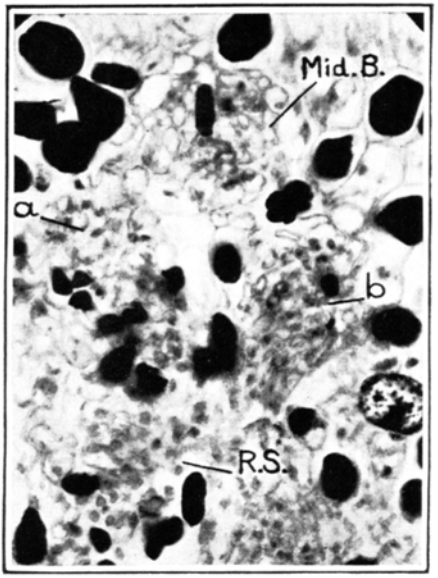
(All figures are from photomicrographs.)

Mid.B.—Midrib Bundle; *a* and *b*—The two bundles forming the vascular supply to a single stamen; St.B.—Fused Stamen Bundle; and R.S.—Receptacular Stele.

Fig. 1. Transverse section of part of the floral receptacle to show the origin of the midrib bundle of the perianth segment with an associated pair of bundles forming the vascular supply to one stamen. ×262·5.

Fig. 2. Transverse section of the midrib part of the perianth at the level of the ovules showing the paired arrangement of the bundles of the stamen. ×315.

Fig. 3. Transverse section at a higher level showing the fusion of the two bundles to form a single large strand for the stamen. ×262·5.



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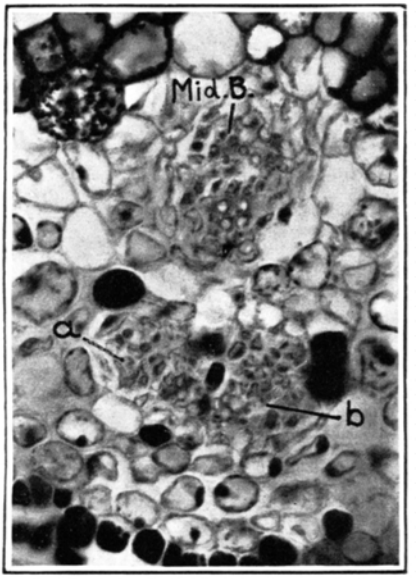

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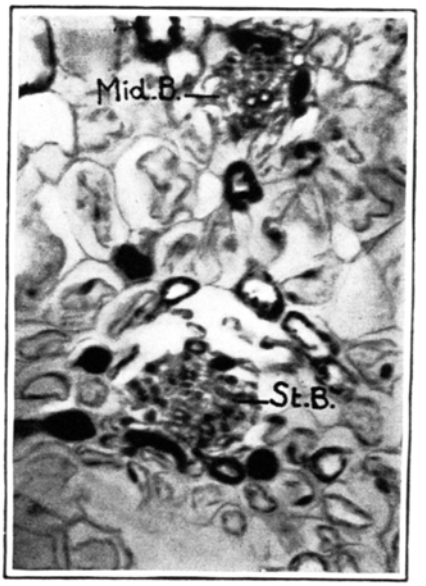

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