THE ANATOMY AND MORPHOLOGY OF THE OPERCULUM IN THE GENUS *EUCALYPTUS*.

PART I. THE OCCURRENCE OF PETALS IN EUCALYPTUS GUMMIFERA (GAERTN.) HOCHR.

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(Plates ii–iii, and two Text-figures.)

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Synopsis.

The nature of the operculum present in the genus *Eucalyptus* has been for many years the subject of a considerable amount of conjecture, and a number of conflicting interpretations as to its morphological nature have been proposed.

In the young buds of *E. gummifera*, four minute imbricate petals have been found, which gradually fuse together to form an inner corolline operculum. This inner operculum remains quite distinct from the outer operculum which is probably calycine in origin.

There is, therefore, a close morphological relationship between the flowers of this species and the two New Caledonian genera *Piliocalyx* and *Acicalyptus*, which may also indicate a close phyletic relationship.

Introduction.

Although a considerable volume of literature dealing with the anatomy of the genus *Eucalyptus* has accumulated, no investigations have yet been carried out on that unique organ, the operculum, most attention having been focussed upon the wood anatomy, leaf structure, etc. This is somewhat surprising, as the exact nature of the operculum has been the subject of a considerable amount of conjecture since 1788 when the genus was first described by L'Héritier.

Historical.

In his original description, and after consideration of the one species only (*E. obliqua*), L'Héritier held the operculum to be corolline in nature. On the other hand, Jussieu (1812) considered that it was formed by the fusion of two bracts. Robert Brown (1814), however, came to the conclusion that the operculum had different origins in different groups of species. He thought that in most species it represented a fusion of the calyx and corolla; in those species with double opercula, the inner structure represented the corolla, and the outer one the calyx; and in the genus *Eudesmia* R.Br., now *Eucalyptus* L'Hér., Series *Eudesmiae* (Blakely, 1934), the operculum was formed from the corolla alone. Hooker (1860) concluded that the operculum was a combined calyx and corolla, but Bentham (1866) was uncertain as to the correct interpretation of the organ, although he considered it to be most likely corolline in nature. He noted the presence of an additional outer operculum in some species, but regarded the nature of this outer organ as doubtful. Bentham thought that this outer operculum would eventually be found to be present in nearly all species but that it was deciduous so early that it was not noticeable in most buds. Maiden (1923) regarded the operculum as corolline in origin except in those species with double opercula where he considered the inner one to be corolline and the outer one calycine. He predicted that eventually all species would be found to have double opercula, the outer calycine one being deciduous very early in most species. Naudin (1883), Deane (1900), Andrews (1913), Hutchinson (1926), Blakely (1934), Rendle (1938), and Osborne (1947) all considered the operculum to be formed from the fused petals, whereas von Mueller (1879–84) concluded that the organ was nearly always calycine in nature. Hardy (1935, 1939), however, thought the operculum was either a modified corolla or a fusion of both calyx and corolla, but he considered the evidence insufficient to determine definitely which interpretation was the correct one.

In view of these conflicting opinions, it was thought that a study of the organogeny of the operculum would definitely decide which of these interpretations should be adopted.

The Present Status of the Problem.

It is apparent from the descriptions of the various species that there can be no general interpretation of the morphological nature of the operculum, and that the species fall roughly into three groups: (1) the Eudesmiae with four minute calyx teeth surmounted by a single operculum; (2) those species with distinct double opercula throughout most or all of the stages of bud development, notably the Corymbosae-peltatae; and (3) those species with a single operculum only throughout most or all of the bud's development. This last group includes the majority of Eucalypt species. These three groups coincide with those of Robert Brown (1814).

There are still good grounds for considering group (1) (Eudesmiae) to be a separate genus closely related to *Eucalyptus*. Also, group (3) is probably derived from group (2) by suppression of one of the opercula or by the fusion of both structures. Consequently, an examination of the opercula in group (2) is the most likely to provide information regarding the fundamental nature of the organ. It may also assist in interpreting the morphological nature of the single operculum in group (3).

Materials and Methods.

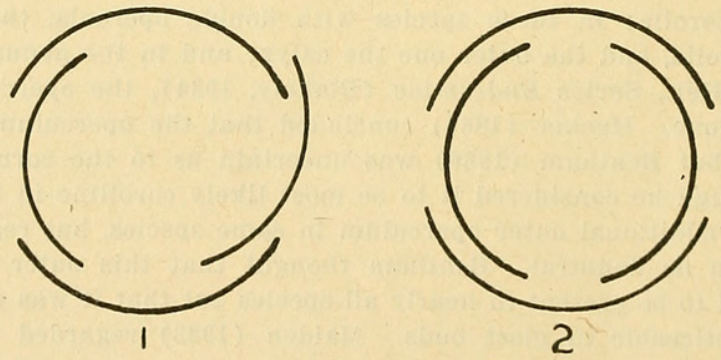
The Bloodwood *E. gummifera* was the first species to be examined as it is a common tree on the Hawkesbury sandstone in the vicinity of Sydney, and so little difficulty was experienced in collecting adequate material. The buds used in this study were collected from three different localities—Avalon, Roseville, and National Park.

The buds at different stages were fixed under reduced pressure in F.A.A. or F.P.A. and embedded in paraffin in the usual way. Staining was carried out with Safranin and Delafield's Haematoxylin using the method of Boke (1939).

The photographs were taken on Kodak Process Pan plates at a magnification of × 30, and have been reproduced at the same magnification.

The Anatomy of the Operculum.

An examination of the very young buds of *E. gummifera* revealed the presence of four minute, imbricate petals, inserted between the staminal ring and outer operculum (Plate ii, figs. 1 and 2). The petals are simple in construction with a uniform epidermis and no cuticle, whilst a constant feature is the presence of many large, well-defined oil glands (Fig. 2). The petals are attached by a broad base, and have a peculiar arrange-



Text-figures 1 and 2.

1. The petal arrangement in *Eucalyptus gummifera*.
2. The expected petal arrangement when the phyllotaxis is opposite and decussate.

ment in that they are not symmetrically imbricate. For example, in the case of three of the petals, the right (or left) edge overlaps the left (or right) edge of the petal next to it, whilst the fourth petal has both right and left edges enclosed by the petals on either side (Text-fig. 1 and Plate iii, fig. 8). As Eucalypts have basically a phyllotaxis of one-half (Jacobs, 1936), one would expect a simple dimerous arrangement as shown in Text-figure 2.

This arrangement described above leads to one petal being folded and enclosed by the other three, so that when the bud is cut transversely, the upper part of the folded petal is sectioned somewhat longitudinally (Plate iii, fig. 7).

As the bud develops, the petals gradually fuse together to form a solid inner operculum, which remains quite separate from the outer conspicuous operculum (Plate iii, figs. 10 and 11). The calyx tube grows faster than both the operculum and the fused petals, and as the proportional volume occupied by the fused petals becomes less and less, they spread out to form an almost flat cap just under the operculum proper (Plate iii, fig. 11). There is always a small space between the two structures and they fall together when the stamens unfold. A comparison of the ratio opercular volume to bud volume in Plate ii, fig. 1 and Plate iii, fig. 11, shows the much more rapid growth of the calyx tube.

Hardy (1935, 1939) has pointed out that the operculum is not a solid mass of tissue as most authors have assumed. Instead, a distinct lobing at or near the apex of the operculum marks the presence of a minute pore, which may either run right through the organ, or only part of the way, according to the species under consideration. Hardy found this pore or traces of it in 55 species, and there seems little doubt that it will be found to be of general occurrence in the genus.

In *E. gummifera* this channel is lined with cuticularized elongated cells, and runs completely through the organ (Plate iii, fig. 9). It nearly always emerges at one side of the operculum near the apex, and rarely through the apex itself (Plate ii, fig. 3; Plate iii, fig. 9). At its upper end the channel is usually three lobed (Plate ii, fig. 4) but lower down it becomes a single elongated slit (Plate ii, fig. 5). It is eventually obliterated as the bud matures.

Discussion.

Angophora is generally considered to be the genus with the closest affinity to *Eucalyptus*, the two genera being the only members of the Subtribe Eucalypteae (Bentham and Hooker, 1862–67). However, in view of the occurrence of four minute petals in *E. gummifera* as described above, it now seems more likely that the genera most closely related to *Eucalyptus* will be found to be the two genera *Piliocalyx* Brong. and Gris., and *Acicalyptus* A. Gray. (Bentham and Hooker (1862–67) placed *Piliocalyx* in the *genera anomala*, and *Acicalyptus* in the Subtribe Metrosidereae.)

Acicalyptus is confined to the Fiji Islands and New Caledonia. Its buds are Eucalypt-like and four-angled, with a beaked operculum which falls off, revealing four minute imbricate petals inserted on the margin of the calyx tube by broad bases. These petals lightly cohere, thus forming a lid which falls in one piece when the operculum is detached. There are numerous inflexed stamens but the ovary has only two loculi. The fruit is unknown (Gray, 1854).

Piliocalyx also possesses an operculum covering four small, unequal imbricate petals which cohere to form an inner operculum. The stamens are indefinite and the fruit is unknown (Brongniart and Gris, 1865). One species is found on Lord Howe Island (von Mueller, 1873), but otherwise the genus is known only from New Caledonia. Both these genera, therefore, bear flowers having a very close morphological relationship to those of *E. gummifera*, and a closer examination of them should prove of great interest, as this similarity in floral morphology may well denote a close phyletic kinship.

It seems likely also that on investigation a situation similar to that described for *E. gummifera* will be found to hold for all other species of *Eucalyptus* with double opercula (mainly the Corymbosae-peltatae), and that by fusion or loss, the single operculum of the majority of Eucalypts has been formed. However, the position of such species as *E. camaldulensis* Dehnh. (Series Exsertae) and *E. microtheca* F. Muell. (Series Buxeales) described by Maiden (1923) as having double opercula, is still obscure.

The presence of the opercular channel described above, may well indicate that the operculum is calycine in nature, especially in view of the lobed nature of the channel near the apex. In this connection it is significant that Hardy (1935, 1939) recorded this channel in two other members of the Corymbosae-peltatae, *E. calophylla* R.Br. and

E. ficifolia F. Muell., the former having four symmetrical lobes at the apex of the operculum, and the latter either four, three or two lobes irregularly arranged, "but approximating to a symmetrical arrangement of four about the axial point". Although Hardy considered the operculum, in the latter species at least, to be a combined calyx and corolla, for simplicity he termed the lobes "petaline vestiges".

However, it has been found (Willis, unpub.) that in *E. ficifolia* (and almost certainly in *E. calophylla*) there are four imbricate petals similar to those reported above for *E. gummifera*, but larger and not completely concrescent. They are pressed closely to the inside surface of the operculum but not fused to it, indicating that the operculum is most likely calycine in nature and is not a composite structure.

The indistinct lobing or lack of lobing, and the incomplete nature of the opercular channel found in such series as the Pachyphloiae and the Piperitales indicates that fusion takes place earlier and earlier in the ontogeny as the species becomes more advanced.

Jacobs (1936) has shown that the phyllotaxis of *Eucalyptus* is basically opposite and decussate. The alignment of juvenile leaves in two rows and the varying arrangements of mature leaves are caused by the growth in length of the stem between each pair of leaves, the twisting of the petioles, and the twisting of the internodes between each leaf pair. Consequently, the petals of a *Eucalyptus* flower would be expected to show two dimerous whorls instead of the arrangement described. The arrangement of the petals of *Piliocalyx* and *Acicalyptus* is at present unknown, but if they show an arrangement similar to that shown by *E. gummifera*, it will be additional confirmation for the close morphological relationship to *Eucalyptus* postulated above for these two genera.

Summary.

1. The presence of four small imbricate petals in *E. gummifera* is demonstrated.

2. The petals have an unusual unsymmetrical arrangement instead of the expected two dimerous whorls.

3. These petals fuse during the development of the bud forming an inner operculum.

4. The outer operculum has a distinct channel or slit running through it which is obliterated before the bud reaches maturity.

5. The relationship between *E. gummifera* and the two genera *Piliocalyx* and *Acicalyptus* is discussed.

6. There is some evidence indicating that the outer operculum represents a fusion of the sepals.

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EXPLANATION OF PLATES II-III.

(All photographs × 30.)

Plate ii.

Fig. 1.—Longitudinal section through young bud showing insertion of petals.

Fig. 2.—Transverse section of young bud just below the level of the stigma showing the four imbricate petals. Two of the petals are beginning to fuse.

Fig. 3.—Transverse section of operculum of young bud showing lateral position of the entrance to the opercular channel.

Fig. 4.—Transverse section slightly lower than Fig. 3 showing triple nature of the channel near the apex of the operculum.

Fig. 5.—Transverse section slightly lower than Fig. 4 showing the opercular channel.

Fig. 6.—Transverse section slightly lower than Fig. 5 showing three imbricate petal segments.

Plate iii.

Fig. 7.—Transverse section slightly lower than Fig. 6 showing the four imbricate petals. The characteristic infolding of the fourth petal beneath the other three is clearly shown.

Fig. 8.—Slightly oblique transverse section at the level of the stigma showing the four imbricate petals.

Fig. 9.—Longitudinal section of bud showing petals and opercular channel.

Fig. 10.—Longitudinal section showing gradual fusion of petal segments.

Fig. 11.—Longitudinal section of mature bud showing the outer calycine operculum, and the inner operculum formed from the fusion of the petals. The irregularly pentagonal objects just below the inner operculum are transverse sections through the filaments of inflexed stamens.