# The Life History of Tupeia Cham. et Schl. (Loranthaceae)

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# SUMMARY.

THE most frequent hosts of the mistletoe Tupeia antarctica are Carpodetus serratus and Nothopanax arboreum. It is substantially light-demanding, and appears to hybridise occasionally with Loranthus micranthus.

Anther development follows the normal Angiosperm pattern, but the gynaecium is entirely solid, with a group of archesporial cells embedded at its base. From these develop unusually elongated embryo-sacs which reach almost to the stigma. Egg apparatus and endosperm nucleus form in the usual manner, but antipodal cells are lacking.

After fertilisation, double suspensors carry the pro-embryo rapidly to the base of the ovary, endosperm formation being delayed until this has occurred. Subsequently by its own growth and that of the endosperm the embryo moves back to the apex

of the ripe fruit.

The seed requires light and moisture for germination. The haustorium is a continuation of the radicle, and establishes connection with the host xylem via the rays. The plumule does not develop until this has occurred.

The present alliance of Tupeia with Loranthus is confirmed

by these observations.

Tupeia is a monotypic genus of the Loranthaceae endemic in New Zealand. The plant was first described by G. Forster (3) as Viscum antarcticum. Schlechtendahl (15) considered that it resembled both Viscum and Loranthus, especially the latter, but made it the type of a new genus because unlike Loranthus it was dioecious. Hooker (7, 8) upheld this genus, remarking that it differed from Loranthus in being dioecious and from Viscum in having perfect stamens. More recently, in their monograph of the Loranthaceae, Engler and Kraus (4) have allied Tupeia with Loranthus and referred to its rounded pollen, obscure calyx and unisexual flowers as distinguishing features. Since the mistletoes show much specialization, particularly in flower and fruit development, the author undertook a detailed study of Tupeia in order to discover any additional noteworthy features. The work was carried out in the Botany Department of Otago University, and thanks are due to Dr. G. T. S. Baylis for assistance and advice.

The general appearance of Tupeia (Figs. 1, 2) is sufficiently described in Cheesman's Flora (2) save that he does not mention the common assumption of a weeping habit. The writer collected in the Dunedin, Kaikoura, Nelson and Gisborne districts, and obtained some evidence of the existence of regional races. Material from Gisborne had more pubescence, broader and thicker leaves and larger inflorescences (6–14 flowers as against 3–5) than South Island collections, and the fruit was dark red, not the usual white or pink (Fig. 3).

Host preferences also varied between districts; in Dunedin and Gisborne Carpodetus serratus was virtually the sole host, but this tree was rarely attacked in Nelson or Kaikoura, where infections were almost exclusively on Nothopanax arboreum. Other plants observed to serve occasionally as hosts were Nothopanax edgerleyi, Coprosma linariifolia and other Coprosma spp., Hoheria lyallii, Pittosporum tenuifolium, Myrsine australis and Rhipogonum scandens.

Thomson (19) has described what appears to be a fertile natural hybrid between Tupeia and Loranthus micranthus, and suggested that the abnormal hermaphroditism which has been recorded in Tupeia (2, 22) might arise through such a cross. The single hermaphrodite plant found by the writer showed some other characteristics suggestive of this origin; its leaves were larger and its anther filaments shorter than usual. Twelve bushes were noted near Dunedin with a leaf form intermediate between those typical of Tupeia and Loranthus in that district, and the fact that none of the females developed normal fruit (in most cases it aborted at the pro-embryo stage) supports the possibility that they were hybrids.

## STEM AND LEAF ANATOMY.

There are few noteworthy features in the structure either of stem or leaf. An early formation of cork in the hypodermis of branches (Figs. 35, 36) and the presence of bordered pits in vessels and tracheids of the secondary wood (Fig. 32) serve to distinguish the genus from Viscum, in which cork is not formed and wood elements bear simple pits only. The phloem, as in mistletoes generally (17, 18) is composed of elongated cells with well developed cytoplasm and nucleus, sieve tubes being absent. The leaf is mesophytic, with a thin cuticle, unsunken stomata and undifferentiated mesophyll. The vein endings are not water storing as they are in Viscum album and species of Loranthus (12, 16). Calcium carbonate crystals are present in the mesophyll, but the deposits of calcium oxalate and silica, common in the parenchyma tissues of the family generally, do not occur in Tupeia.

## THE MALE FLOWER.

The flowering shoots arise as axillary or terminal buds protected by several pairs of scales (Fig. 10). In Dunedin these open late in July. Between one and three pairs of small leaves emerge, and on expanding reveal the terminal raceme of 3-5 flowers. The flowers open towards the end of August with their pollen already ripe (Fig. 9).

The male flower (Figs. 7, 11) is about 4 mm. in diameter and pale green in colour. Its perianth of four narrow members shows so slight a basal union that it is better regarded as free (8) than as forming a perianth tube (2). The anthers are oval and basifixed to well defined filaments, each of which fuses with the base of the perianth member opposite which it stands (Fig. 11). The pollen sacs dehisce inwards through two longitudinal slits, and pollination appears to be effected by small flies attracted by a honey disc at the centre of the flower.

In some Loranthaceae development and mature structure of the anther is unusual (9, 14, 17, 18) but in *Tupeia* it follows the normal course in Dicotyledons save that the interlocular strands of tissue do not break down completely prior to dehiscence (Figs. 12, 13). When shed the pollen grain is rounded, with an irregular surface, and 2–4 pores. It is binucleate and germinates readily in glucose solution.

The vascular system of the flower affords no evidence of previous existence of a greater number of parts. The pedicel contains four strands. One enters each perianth member and gives off a branch to the filament.

### THE FEMALE FLOWER.

The female inflorescence is born in the same manner as the male, but the flowers are only about 2 mm. in diameter (Figs 6, 8). The perianth is of four narrow segments, regarded by Cheeseman (2) as free tips of members adnate to the ovary, and by Hooker (8) as free petals above an inferior ovary. The style is stout and straight and the capitate stigma is obscurely lobed (Fig. 14). The whole style is deciduous and falls about a fortnight after the perianth is shed—i.e., about four weeks after the flower opens.

The ovary of most Loranthaceae contains a chamber, but one of the characters dividing the family from the Santalaceae is the lack of normal ovules therein. Instead there is a basal papilla, the mamelon, in which several embryo-sacs develop. This mamelon fills the ovary to a varying degree. When it is large it tends to fuse with the sides of the chamber, and in extreme cases the entire chamber is obliterated. The extent to which the mamelon fills the ovary is not necessarily a generic character. Thus, though in Arcenthobium (9, 18) the mamelon is always free from the ovary walls, in Loranthus (5, 6, 21) a series can be traced through forms in which there is a temporary chamber filled by later growth of the mamelon, as in L. spaerocarpus and L. europeus, to L. scurrula, where the ovary is always a solid body

Tupeia proves to be like L scurrula and the New Zealand species of Korthalsella (17) in possessing no trace of an ovary chamber. Internal differentiation in the gynaecium is first apparent 8 or 9 weeks before the bud opens. A longitudinal section (Fig. 14) then reveals a column of large starch containing cells leading down the style and through an entirely solid ovary to a group of 4-12 archesporial cells at its base. Of these only three or four complete development into embryo-sacs. This process begins presumably with meiosis, but the chromosomes were too small for this to be confirmed by direct observation. Of the first pair of daughter cells only the upper undergoes the second division, so that a tier of three cells replaces the usual megaspore tetrad (Figs. 15, 16, 17). It is the uppermost megaspore that develops into the embryo-sac. It immediately begins to elongate upward through the starch column until it reaches more than halfway up the style (Fig. 18). There is also some extension downwards which crushes the sister cells, but it is then halted by a funnel shaped sheath of collenchyma which has developed below them. A nuclear division occurs as cell elongation begins, and one of the daughter nuclei moves to the slightly dilated stylar end of the sac and here divides in the usual way to produce an egg, two synergids and one component of the fusion nucleus (Fig. 19). The activity of the nucleus remaining at the base of the sac has not been so clearly followed, and is probably limited to a single division, since no distinct antipodal cells are formed (Fig. 20). However, there can be little doubt that it contributes its component to the fusion nucleus as this appears to be diploid, being large and possessing two nucleoli, whereas all others have one. The fusion nucleus is situated a little below the egg apparatus in the ripe sac (Fig. 19).

When the flower opens it contains, therefore, three or four embryo-sacs which, apart from their unusual length and position, are abnormal only in lacking antipodal cells. This is by no means an uncommon feature in many families (14, 20). Elongate embryo-sacs are usual in the Loranthaceae, and the amount of upward and downward growth may vary even within one genus—e.g., Arceuthobium (9). Upward extension appears to predominate in Loranthus, but in no species does it extend quite so close to the stigma as in Tupeia, though in L. pentandrus (20) it does reach halfway up the style. Both Arceuthobium and Viscum have well defined antipodal cells, but their egg apparatus is less conspicuous than that of Tupeia.

## FERTILISATION AND FRUIT DEVELOPMENT.

Mistletoes in which there is only moderate elongation of the embryo-sac are usually pollinated in the winter and fertilised in the spring. In some cases the embryo-sac has just begun development at the time of pollination (5, 9). In Tupeia the ends of the mature sacs lie close below the stigma when the pollen arrives and fertilisation follows quickly. The process was not actually observed.

The first division of the zygote is apparently vertical, since the suspensor is double. It becomes transversely sub-divided and greatly elongated, so that it carries the pro-embryo down out of the style before this is shed (i.e., within 3-4 weeks of pollination) and lodges it at the base of the sac within the collenchyma sheath. The endosperm nucleus also moves clear of the style (Figs. 21, 22).

The pro-embryo at this stage is a tier of three cells. Further growth comes from the lowest of these cells, the upper ones merely accommodating themselves by longitudinal divisions to the increasing breadth of the embryo, and ultimately becoming crushed with the suspensor against the developing endosperm. This tissue begins to form as scattered cells on the side of the sac after elongation of the suspensor is complete (Fig. 23).

Since there is more than one embryo-sac in the ovary, normally more than one pro-embryo reaches the collenchyma sheath (Fig. 22), but usually soon after that all but the most vigorous one have aborted. However, occasional fruits do mature two or even more embryos. These lie in a common endosperm mass, so that it is likely that even in fruits with one embryo this tissue is compound and derived in part from endosperm nuclei of sacs that have aborted at a late proembryo stage. There is no evidence that embryos themselves coalesce as has been recorded in *Loranthus globosus* (5).

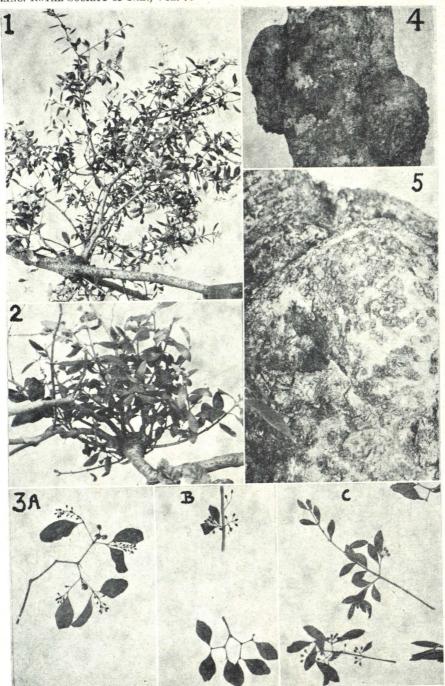


Fig. 1—Young bush of Tupeia antarctica upon Carpodetus serratus, × 1/12. Fig. 2—A young infection showing how the host stem is encircled. × ½. Fig. 3—Herbarium material. A—Gisborne, B—Nelson, C—Dunedin. × ½. Fig. 4—Old galls on a Carpodetus trunk where Tupeia infections have been shaded out.

Fig. 5—Surface of an old gall. Two feeble shoots of Tupeia persist in the lower Photographs 4 and 5 taken by P. J. Brook. left-hand corner.

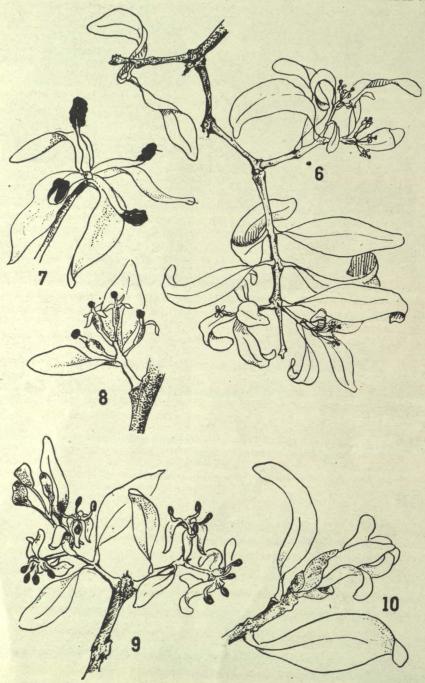


FIG. 6—Female flowering branch. Natural size.

FIG. 7—Male flower. × 14.

FIG. 8—Female inflorescence. × 5.

FIG. 9—Terminal male inflorescence. × 5.

FIG. 10—The leaves of a flowering shoot emerging from the protecting scales. × 6.

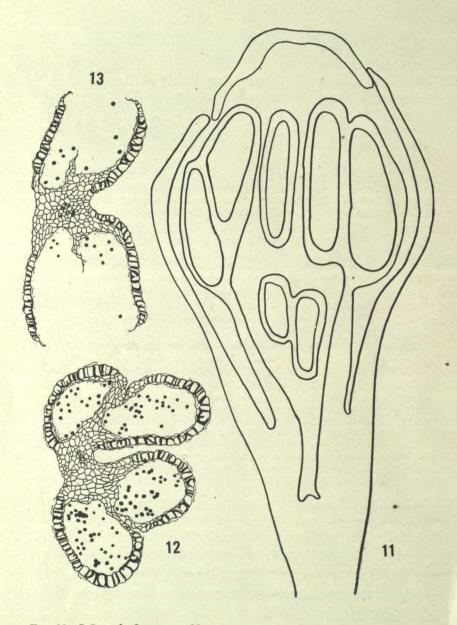


Fig. 11—L.S. male flower.  $\times$  38.

Fig. 12—T.S. anther, showing the incomplete breakdown of interlocular strands prior to dehiscence.  $\times$  85.

Fig. 13-T.S. dehisced anther, × 85,

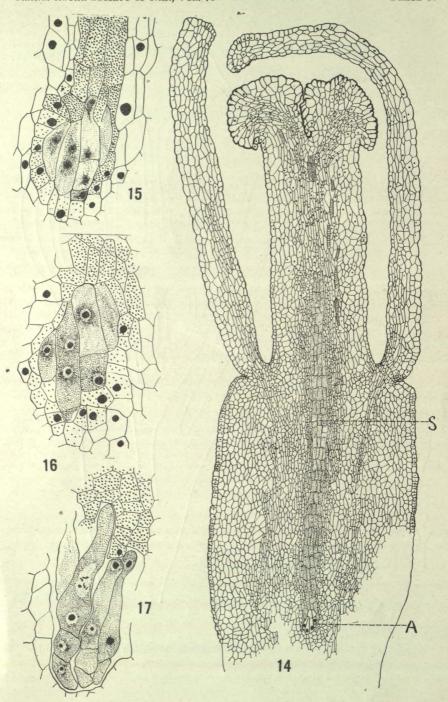


Fig. 14—L.S. young female flower. A group of archesporial cells (A) lies at the base of the starch column (S) in an entirely solid ovary.  $\times$  60.

Figs. 15, 16 and 17—Archesporial cells in process of division each into a tier of three cells, of which the uppermost continues development to form the embryo-sac, × 420.

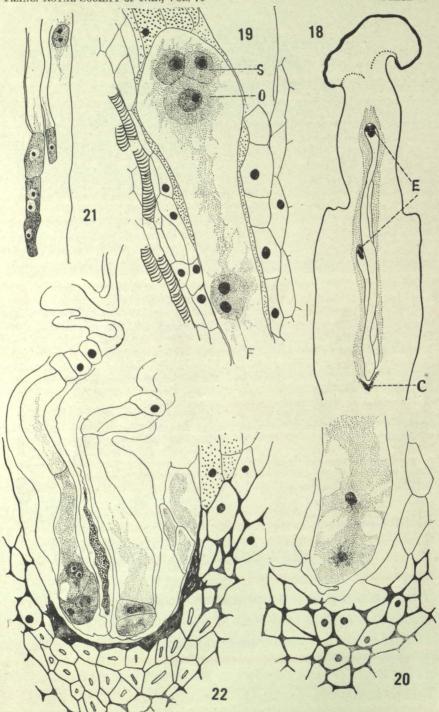


FIG. 18—L.S. female flower showing elongated embryo-sac with egg apparatus (E) at their stylar ends, and the collenchyma sheath (C) at their base. × 37.

FIG. 19—Dilated stylar end of an embryo sac showing the egg (O), synergids (S) and fusion nucleus (F). × 420.

FIG. 20—Lower end of embryo sac. × 420.

FIG. 21—The pro-embryo being carried down the style by the suspensor, and the fusion nucleus accompanying it. × 420.

FIG. 22—A group of pro-embryos that have reached the collenchyma sheath, the most vigorous already developing to the detriment of the remainder. × 420.

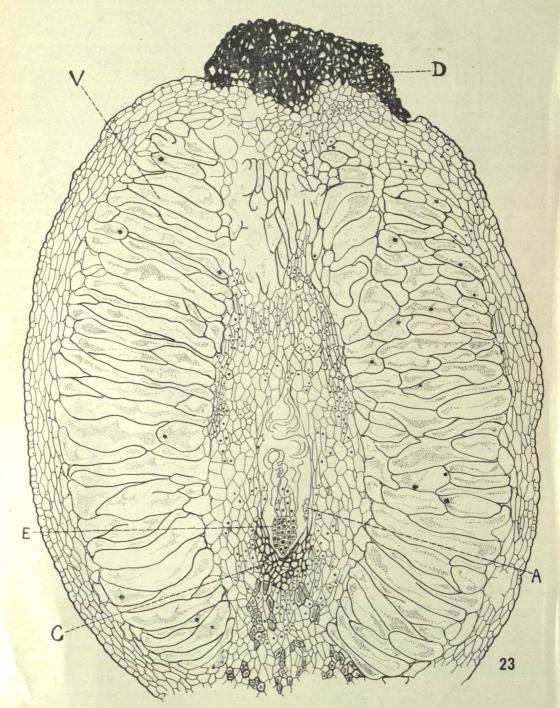


Fig. 23—L.S. developing fruit. V, viscous tissue; D, stylar disc; C, collenchyma sheath; E, embryo; A, endosperm cells.  $\times$  60.

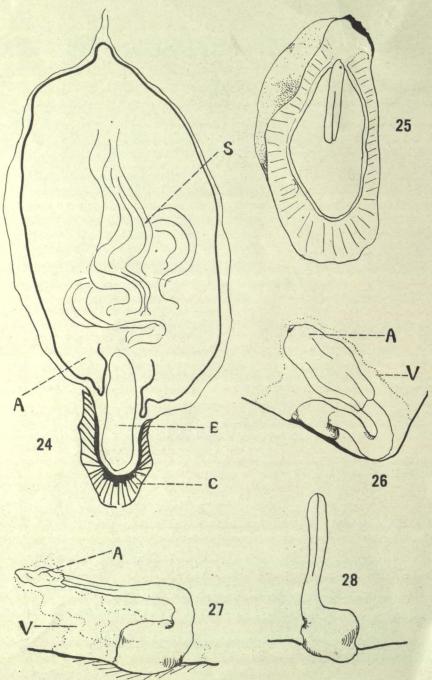


Fig. 24—L.S. developing fruit. The embryo (E) is being shouldered out of the collenchyma sheath (C) by developing endosperm (A). Remains of suspensors (S) still evident. × 128.
Fig. 25—L.S. ripe fruit. The embryo (E) lies in the upper half of the endosperm (A) and viscous tissue (V) forms the bulk of the fruit wall. × 12.
Figs. 26, 27 and 28—Seedling establishment. A, endosperm; V, adhesive coating. × 16.

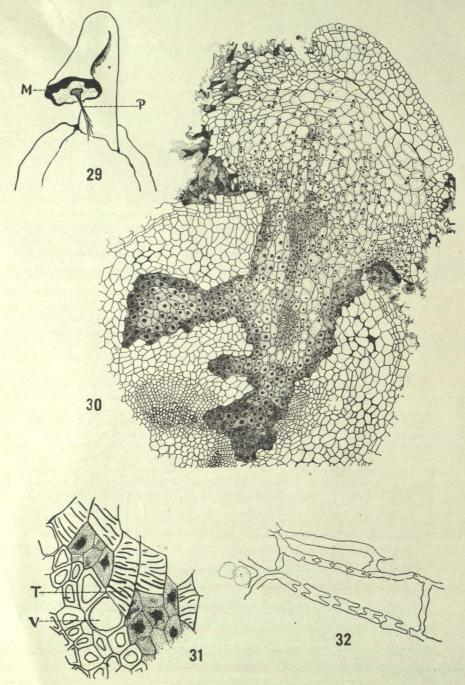


Fig. 29—Seedling detached from the host just after the formation of the penetrating strands (P). Cementing material (M) surrounds the concave haustorial disc. × 17.

Fig. 30—A young haustorium invading a twig of Carpodetus. × 57.

Fig. 31—Tracheids of a haustorium (T) in contact with vessels (V) of Carpodetus where these adjoin an invaded ray. × 420.

Fig. 32—Tracheid in L.S. to show bordered pits. × 800.

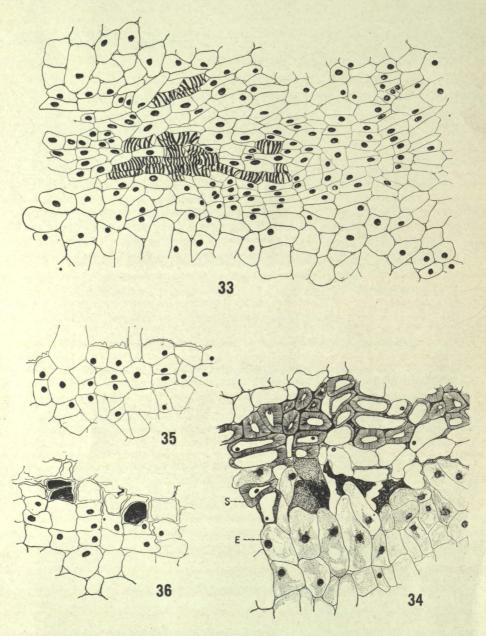
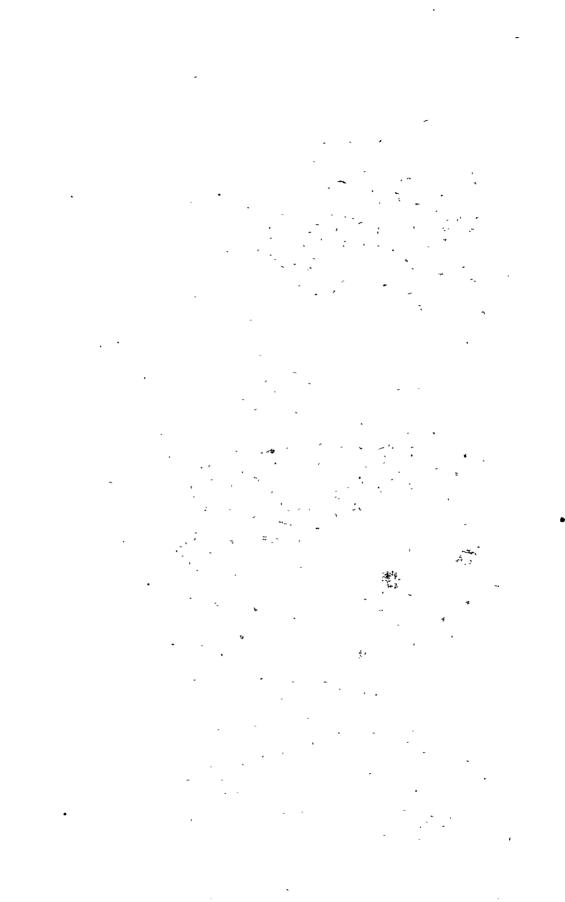


Fig. 33—L.S. advancing haustorium showing tracheids differentiating from dividing cells. × 233.

Fig. 34—The epidermis (E) of a haustorium against a group of stone cells (S) in the cortex of Carpodetus. The stone cells are undergoing solution.  $\times$  280.

Figs. 35 and 36—T.S. young stems of *Tupeia* showing cork cambium arising in the subepidermal layers and producing cork.



In the later stages of development the embryo grows actively at the radicle end. It extends up into the body of the ovary, and is shouldered out of the collenchyma sheath by the expanding endosperm (Fig. 24), which comes to surround it completely. By the time the embryo has developed cotyledons it is lying not at the base but in the centre of the fruit, and its upward movement continues.

Meanwhile the viscous tissue characteristic of Loranthaceous fruits (5, 10, 13, 17, 18, 20, 21) has been developing. It is discernible soon after fertilisation as a cylinder of cells extending from the level of the collenchyma sheath nearly to the style base. These cells enlarge greatly, especially radially, and constitute the bulk of the fruit wall (Fig. 23). At the apex of the fruit the viscous tissue cylinder folds inwards and envelops each of the four vascular strands in a separate sheath.

The mature fruit is ovoid and about 4 mm. long. The style base forms an apical disc which plugs the apex of the old embryo-sac cavities. Beneath the epidermis are four or five layers of parenchyma, then disorganised viscous tissue separated by a further layer or two of parenchyma (often reduced to a membrane) from starchy endosperm, in the apical half of which lies the embryo in an axial position (Fig. 25).

In comparison with other members of the Loranthaceae, fruit formation in *Tupeia* shows no unusual features. A double suspensor has been recorded in several species of *Loranthus* (20, 21) and a collenchyma sheath at the base of the gynacium seems to define always the downward limit of embryo-sac extension when these are of the elongating type (6, 20, 21). The migration of the embryo to the base of the ovary through expansion of the suspensor and its subsequent return to the apex of the fruit by its own upward growth aided by pressure from the endosperm is closely paralleled in *Loranthus sphaerocarpus* (21). but in this plant the formation of endosperm begins relatively early, the suspensor forcing the pro-embryo downward through the already developing endosperm tissue.

#### DISSEMINATION AND GERMINATION.

The fruit of Tupeia ripens between August and November, about a year after the flower opened, as is usual in the Loranthaceae (1, 21). It is occasionally forced from its pedicel by swelling of the viscous tissue, but normally it awaits dislodgement by some external Birds are the natural means of seed dispersal. swallow the entire fruit and deposit embryo and endosperm undamaged in their excrement. In this way several are placed together, but on germinating, one seedling appears to gain ascendancy over the rest. The mixed male and female bushes that occasionally occur. however, are likely to be the result of more than one seedling surviving from such a group. Whereas bird droppings containing Tupeia seed were only seen occasionally, single seeds were commonly found adhering to higher branches near a parent bush. Apparently most of the fruit is consumed by small birds which do not swallow the seeds but, when these adhere to their bills, wipe them off against a branch.

The embryo has no rest period, and if the fruit is squeezed the unintegumented seed slips out coated with mucilage derived from the viscous tissue, and the embryo will continue development until the endosperm is exhausted provided light and moisture are available. In darkness or on a dry surface there is no further growth. At approximately 25° C. the endosperm was exhausted in 14 to 20 days, and the embryo would not develop further even if salts and sugar were supplied. Out of doors growth was much slower.

The entire course of seedling establishment has been followed on Carpodetus branches under natural conditions at Dunedin. The seed is attached to the bark by its adhesive coating, which imbibes water and helps to maintain the necessary moisture about it (Fig 26). The radicle already protrudes from the endosperm, and like all other parts of the embryo contains chlorophyll. It is apparently insensitive to gravity, but turns away from light and circumnutates as much as its limited length (2–5 mm.) allows. Internally it develops a diarch stele without, however, the well defined endodermis usual in roots. Its tip does not carry a root cap.

When the apex of the radicle touches the bark it expands into a disc, the marginal cells of which excrete a cementing fluid. The disc becomes concave, and from its centre a few filamentous strands enter the bark and probably facilitate the subsequent entry of the haustorium (Fig. 29). This is initially a club-shaped growth from the centre of the disc which has a meristematic surface. It soon develops a number of lobes which extend along the cortex and down the rays of the host (Fig. 30). Strands of tracheids differentiate along the axes of the haustorium and its lobes and terminate against *Carpodetus* vessels where these adjoin the invaded rays (Fig 31). A water conduction system is thus established between parasite and host. No phloem forms in the haustorium. The tracheids are short and spirally or reticulately lignified. They are flanked by dividing cells from which further tracheids develop (Fig. 33).

The stimulus which induces disc and haustorium to form appears to be purely mechanical, both develop on unfavourable hosts and even against the style base or endosperm of the fruit if it is germinating unshed. In such cases the haustorium, failing to penetrate, becomes an irregular mass of tissue. Where penetration occurs it is mainly by solution of host tissues, a process which becomes very obvious when one of the groups of stone cells in the cortex of Carpodetus is encountered (Fig. 34).

It requires about six months for the haustorium to establish connection with the host xylem. Only then does the hypocotyl straighten and withdraw the cotyledons from the shrunken endosperm and the plumule begin to develop (Figs. 27, 28). The first pair of leaves are close to the cotyledons and short lived. The primary shoot grows steadily for a time, but it is usually superseded by others developing from the attaching disc, which slowly thickens and extends round the stem as the haustorium within girdles the cortex (Fig. 2). The branch becomes swollen partly with the bulk of the haustorium and partly through hypertrophy of the invaded cortex. Its distal length may die away or develop a mass of twiggy reversion shoots. The

parasite remains healthy only as long as it is exposed to fairly strong light, and if later growth of the host or surrounding vegetation shades it heavily it dies away, leaving a large gall on which a few feeble shoots of *Tupeia* sometimes persist (Figs. 4, 5).

The seedling and process of establishment differ in no important way from Loranthus (1, 10) except for the fact that Loranthus forms secondary roots which spread over the surface of the bark and produce secondary haustoria and additional leafy branches at considerable distances from the original infection. In Tupcia the branches are always closely set about the original point of entry.

## DISCUSSION.

This investigation reveals nothing that is inconsistent with a close alliance of *Tupeia* and *Loranthus*. The details of ovary and embryosac show its affinity with those species in which the ovary chamber and mamelon are evanescent or no longer defined, and the embryosacs extend substantially into the style. On the other hand the formation of cork, the bordered pits in the wood, the orthodox anther and very long suspensors emphasise the comparative remoteness of its relationship with *Viscum* in which no cork is formed, woodpitting is simple, suspensors may be lacking and the anthers are fused with the petals and subdivided into more than four chambers.

Nothing can be added to the morphological features which at present separate *Tupeia* from the small flowered species of *Loranthus*, viz.:

Loranthus
Flowers hermaphrodite.
Obscure calyx.
Filaments markedly epipetalous.
Spread by means of laterals bearing new branches and haustoria.

Tupeia
Flowers usually unisexual.
No calyx.
Filaments slightly epipetalous.
One point of infection only.

The fact that Loranthus micranthus and Tupem antarctical hybridize emphasises their affinity, but it is interesting to note that the occurrence of hybrids did not prevent Engler and Kraus from raising to generic rank the sections of Elytranthe to which E. colensoi and E. flavida were referred (4)

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