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THE POLLEN-STIGMA INTERACTION IN THE GRASSES. 7. POLLEN-TUBE GUIDANCE AND THE REGULATION OF TUBE NUMBER IN ZEA MAYS L.

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SUMMARY

The stigma of *Zea mays* can support the hydration and germination of considerable numbers of pollen grains, yet, in general, only one tube enters the micropyle to effect fertilisation. The numbers are reduced at various points in the pollen tube pathway, (1) by competition on the receptive trichomes and in the transmitting tracts, (2) by elimination of late-entering tubes at the stigma abscission zone, (3) at a constricted zone of the transmitting tracts in the upper ovary wall, and (4) in the vicinity of the micropyle.

The control imposed at the abscission zone is higly effective. The entry of the first few tubes into the ovary wall induces a loss of turgidity of the cells of the zone and a disruption of the vasculation, and thereafter further tubes are irrevocably blocked in the main body of the stigma. The device maximises the period available for pollen capture and yet provides for the strict regulation of the number of tubes ultimately entering the ovary once pollination has taken place.

Pollen tubes enter the ovary cavity by breaking through the inner epidermis of the ovary wall, which, like the contiguous inner integument, bears a thin discontinuous cuticle. Further passage is between the inner epidermis and the integument. Growth is irregular, but not wholly random, the tubes orienting themselves in the general direction of the micropyle following lines demarcated by the elongated cells of the epidermis and the integument.

Almost throughout the pollen tube pathway, the cells in the vicinity show characteristics suggestive of a secretory function. The significance of this for pollen tube nutrition and guidance is briefly discussed.

1. INTRODUCTION

The individual stigma ('silk') of *Zea mays* is capable of capturing large numbers of pollen grains, and although with heavy pollination the proportion of grains germinating and producing tubes successful in gaining entry into the stigma may be quite low, substantial numbers of tubes may still reach the transmitting tracts. Yet, in general, only one tube ultimately enters the micropyle to release the gametes. MILLER (1919), one of the most perceptive of the earlier students of the reproductive system of *Zea*, recorded that in nearly a hundred observations no more than a single tube was actually seen by him in the ovary cavity. We have not found quite such a radical degree of elimination, but there is no

doubt that a very severe weeding out of tubes does occur before the final phase of growth within the ovary cavity. The nature of this process is of considerable interest, firstly because should it act selectively upon *Zea* tubes of different genotypes it could affect segregation ratios, and secondly because it could act as a control over the penetration of alien tubes and so contribute to species isolation.

Observations on various Hordeae (review, J. HESLOP-HARRISON 1979) have shown that the structural organisation of the grass stigma – and especially the restrictions imposed by the carrying capacity of the transmitting tissues – can lead to a thinning out of the populations of pollen tubes passing towards the ovary. Undoubtedly this must also be a factor in eliminating tubes in the *Zea* stigma; but in this species the transmitting tracts are comparatively massive, and can provide passage almost to the stigma base for a substantially greater number of tubes than eventually do achieve entry into the ovary cavity. The main sites of elimination must therefore be in the latter part of the pollen tube pathway. This has been described in general terms in previous accounts dating from those of WEATHERWAX (1917) and MILLER (1919), but it has not hitherto been traced in detail in maize, nor for that matter in any grass species.

A further problem concerns the guidance of the pollen tubes in the latter part of the pathway into the ovule. In the stigma itself, the direction of growth of the pollen tubes is determined in the first instance by the conformation of the basal cell complex of the receptive trichomes, the architecture of which establishes that entering tubes grow into the axis with their tips oriented towards the ovary (Y. HESLOP-HARRISON, REGER & J. HESLOP-HARRISON 1984b). Tubes passing directly into the stigma axis from pollen that has germinated in axial sites rather than on the receptive trichomes escape this control, and grow either towards the base or the apex of the stigma. Once in the axis, correctly oriented tubes make their way into the transmitting tracts, crossing the cortex in a stepwise traverse across successive cell files. No special structural adaptation directing the tubes towards the tracts could be found in the cortex of the stigma, but their progression is generally quite positive, with little evidence of random searching. This leaves open the possibility that the tubes seek the tracts in consequence of a chemotropic stimulus, although no direct evidence of this is as yet available. Because tubes from trichome sites tend to retain their initial direction of growth in crossing the cortex, they pass into the tracts oriented towards the ovary. The transmitting tissue is composed of elongated, fusiform cells with large intercellular spaces, and in the tracts the tubes grow rapidly, following these spaces normally without change of direction. The occasional tubes that do happen to be disoriented when they enter the tracts grow either in the basipetal or acropetal directions, indicating that there can be no chemotropic control in the tracts themselves.

We may conclude, then, that *Zea* pollen tubes entering the *Zea* stigma in the customary manner through receptive trichomes are chanelled into the direction of the ovary mainly by mechanical constraints, although they may possibly be subject to chemotropic control for one part of the pathway, that across the

stigma cortex. There remains the questions of how the micropyle is located at the other end of the pathway. This matter as it affects angiosperms in general has been a topic for speculation for many years. Whereas STEFFEN (1951) and others have argued that in some plants the guidance must be purely mechanical, experimental evidence from other species – notably that adduced by ROSEN (1961) and WELK, MILLINGTON & ROSEN (1965) from observations on *Lilium* – suggests that diffusible chemotropic factors originating from the vicinity of the micropyle are responsible for the final directionality of growth. These factors could be the products of the tissues of the nucellus, or of the adjacent integuments. However, the possibility that the embryo sac itself produces chemotropic factors has been accepted quite widely, and VAN DER PLUIJM (1964), following one of the earliest electron-microscopic studies of embryo sac structure, pointed to the synergids as possible sources. The filiform apparatus of the synergid has characteristics of a transfer cell wall, suggesting a secretory function at some stage of development. The fine-structural investigations of DIBOLL & LARSON (1966) and DIBOLL (1968) showed that the synergids of *Zea* have many of the cytological features of secretory cells, with numerous organelles and abundant vesiculate endoplasmic reticulum concentrated at the micropylar pole in the vicinity of the filiform apparatus; but direct evidence of secretory function for these cells is still wanting. In species of *Paspalum,* CHAO (1971, 1977) has shown that all of the cavities in the vicinity of the micropyle – between the nucellus and the integuments, between the integuments themselves and between the integuments and the ovary wall – contain water-soluble polysaccharide, through which the pollen tubes grow in their passage to the embryo sac. CHAO (1977) suggested that this may be a secretion product of the nucellar or integumentary cells, or may arise in part from the dissolution of neighbouring cell walls during the final maturation of the ovule. He further speculated on the possibility that the material might have some chemotropic function. We have shown that aqueous, polysaccharide-containing secretions occur almost throughout the pollen-tube pathway in the *Zea* stigma (Y. HESLOP-HARRISON, REGER & J. HESLOP-HARRISON 1984a), being present in the intercellular spaces of the receptive trichomes, in the stigma cortex and copiously in the transmitting tracts, where the quantity is reinforced during the passage of the pollen tubes by further transfer from the cells of the tract, possibly occasioned by the pressure of the tubes themselves. The material undoubtedly contributes to the medium through which the pollen tubes grow, and it is noteworthy that cytochemical evidence shows that protein is also present. The most likely role of the polysaccharide, and presumably of other carbohydrate fractions yet to be identified in the intercellular secretions, is to form a source of metabolites for the extending tubes. The calculations given in an earlier paper (Y. HESLOP-HARRISON *et al.* 1984b) indicate that the endogenous reserves of the *Zea* pollen grain could support no more than 2 cm of tube growth, after which there would have to be recourse to exogenous substrate encountered by the tube during its extension.

Attributing a nutritional role to the secreted polysaccharide in the pollen-tube pathway does not of course imply that chemotropic factors could not also be

present. To provide the conditions needed for a chemotropic response, however, these would have to be distributed in such a manner as to form a concentration gradient in the vicinity of the micropyle, not distributed ubiquitously in the pollen-tube pathway in the manner of the polysaccharide component. The expectation is that such a gradient would arise by the release of the product from a localised source near, or at, the actual target of growth – in this instance, the tip of the embryo sac. This is the site of the synergids, pointing again to their possible function in the final stage of tube guidance.

In the present study, we have been concerned with identifying features of the *Zea* pistil that might have some function in regulating the number of tubes effecting passage from the stigma into the ovary, and also with gaining some indication of what factors might be involved in providing guidance for the tubes over the last part of their journey to the micropyle. We also give some account of the presumed secretory tissues in the tube pathway. Fine-structural details of these tissues will be described elsewhere.

2. MATERIALS AND METHODS

The observations were made on greenhouse-grown plants of Hybrid 304C, Pioneer Overseas Corporation. Controlled pollinations were carried out on female inflorescences *in situ*, or on excised segments of the cob held *in vitro* at *c.* 24 °C and 70–80% RH. Pollen was collected for immediate use from plants of the same line, or from a second stock, Pioneer Hybrid 3147.

Dissections were carried out on excised ovaries of the required ages in the fresh state, or after fixation for 3–4 h in 1.5% glutaraldehyde in phosphate buffer at pH 7.2 with 8% sucrose. Material for sectioning was fixed in the same manner and transferred through an alcohol series into HEMA resin (TAAB Laboratories). After polymerisation and heating to the required hardness, the blocks were sectioned with glass knives at 1.5–2 μm. The main cytochemical methods applied to the resin-embedded material have been described in earlier papers (Y. HESLOP-HARRISON *et al.* 1984a and references therein).

3. OBSERVATIONS

3.1. The stigma abscission zone

Following a successful pollination, the stigma is ultimately shed (*fig. 3*). The detachment occurs in the trichomeless zone of the stigma base, which in the lines examined extends over a distance of *c.* 5 mm (Paper 5). The site is not demarcated morphologically in the unpollinated stigma (*fig. 1*), nor have any characteristic features of the tissues of the zone yet been identified prior to pollination. The first evidence of a reaction in the abscission zone was observed within 6 h of the application of viable, compatible pollen to the trichome-bearing zone of the stigma, the cortical cells losing turgidity (*fig. 2*). In 24 h, the zone was flaccid, and the vascular tissue and associated transmitting tracts partly dis-

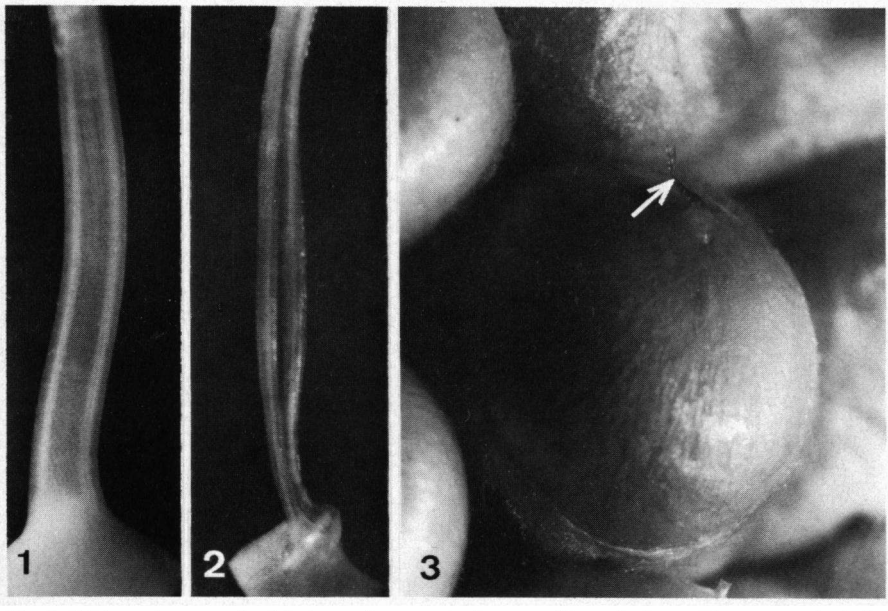


Fig. 1. Trichomeless stretch of the base of a receptive but unpollinated stigma. The segment includes the prospective abscission zone. × c. 18.
Fig. 2. Segment of a stigma base corresponding to that of *fig. 1*, 6 h after pollination, showing the loss of turgidity in the abscission zone. × c. 18.
Fig. 3. Mature fruit after the abscission of the stigma (arrow). × c. 7.5.

rupted (*fig. 4* & inset). Cytological examination showed that these changes in the tissues of the abscission zone followed upon the advent of the first pollen tubes into the upper ovary wall. In the stigma illustrated in *fig. 4*, seven tubes were observed in the transmitting tracts at the points of entry into the ovary wall proximal to the abscission zone. The numbers in the tracts distal to the zone could not be established with certainty, but the total exceeded 30. The elimination had occurred through the arrest of the tubes in the part of the stigma labelled 'Iz' in *fig. 4*, either by the cessation of growth (*fig. 5*), or by enlargement of the tip, followed by bursting and the coalescence of the contents of several tubes to form an embolism in the transmitting tract. A few tubes were found to have made random excursions into the cortical tissues of the stigma after having been displaced from the tracts, but none by-passed the abscission zone by this manoeuvre.

The abscission zone is therefore an effective barrier to the entry of supernumerary tubes into the ovary. The minimal number of tubes required to initiate the changes that block further passage through the zone remains to be established, but the observations so far made indicated that the penetration of the first 5–10 tubes into the ovary wall may well suffice.

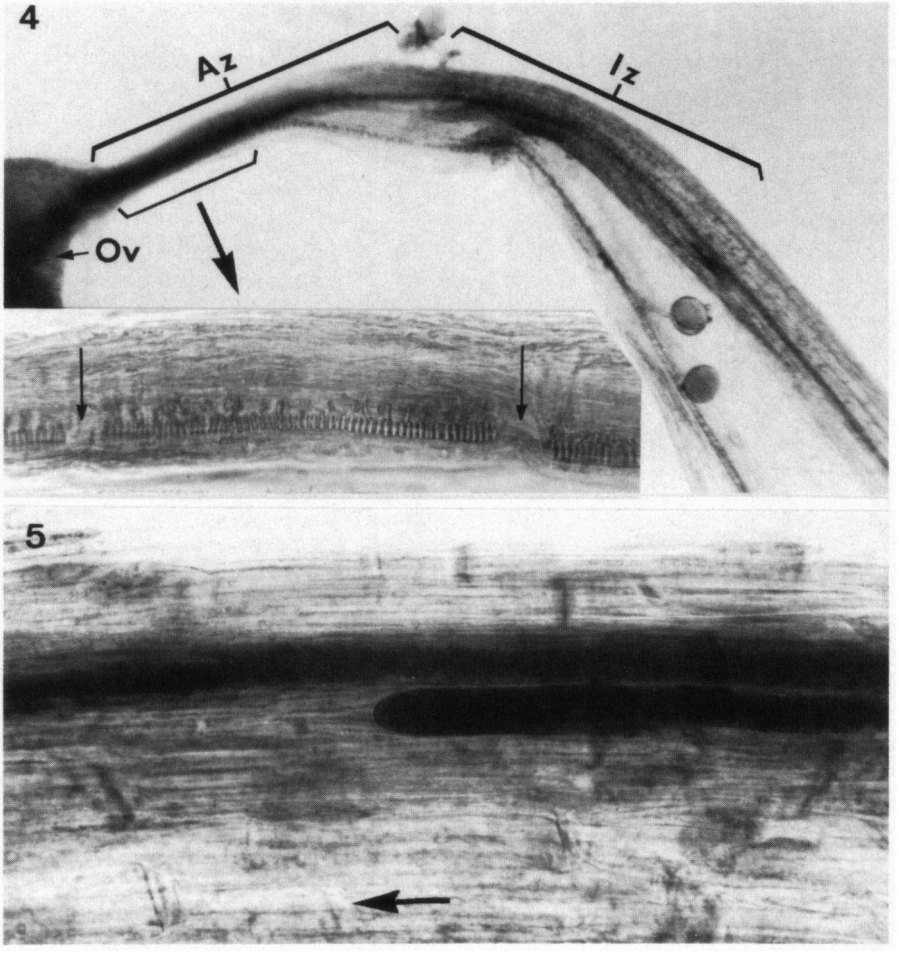


Fig. 4. Whole mount of a stigma base 24 h after pollination (glutaraldehyde fixation, followed by post-fixation in 1% OsO_4 in 0.1 M phosphate buffer at pH 7.0 for 2 h at c. 4 °C). The pollination was carried out *in vitro* on a segment of the ear from which the ensheathing bracts had been removed, and pollen was applied along most of the length of the stigma. In the abscission zone (Az) the vasculation is already disrupted, as may be seen at the arrows in the inset. In this preparation, 7 tubes were observed on the ovary (Ov) side of the abscission zone, with the majority blocked in the zone Iz (cf. *fig. 5*). As the micrograph shows, pollen grains applied to stigma in the trichomeless part germinated freely in the conditions of the experiment (c. 24 C°, 70–80% RH). × c. 55; inset, × c. 320.

Fig. 5. Detail of the zone Iz of *fig. 4*, showing tubes arrested in the transmitting tract distal to the abscission zone. The arrow indicates the direction of the ovary. × c. 600..

3.2. The upper ovary, integuments and nucellus

In the accounts of WEATHERWAX (1917), MILLER (1919), RANDOLPH (1936) and others, it has been the convention to view the pistil in longitudinal section in a plane radial to the rachis. For convenience of reference, the longitudinal axis

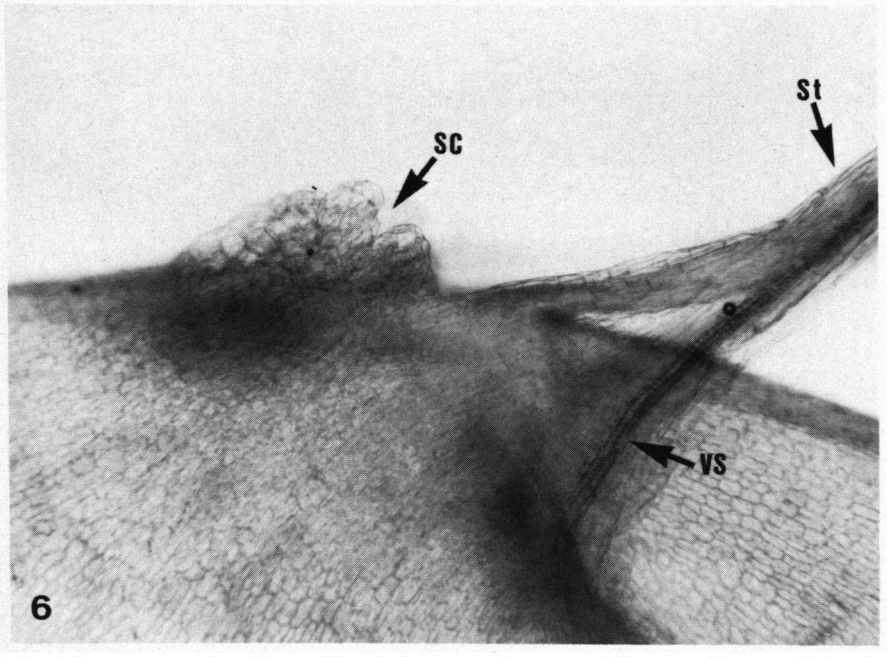
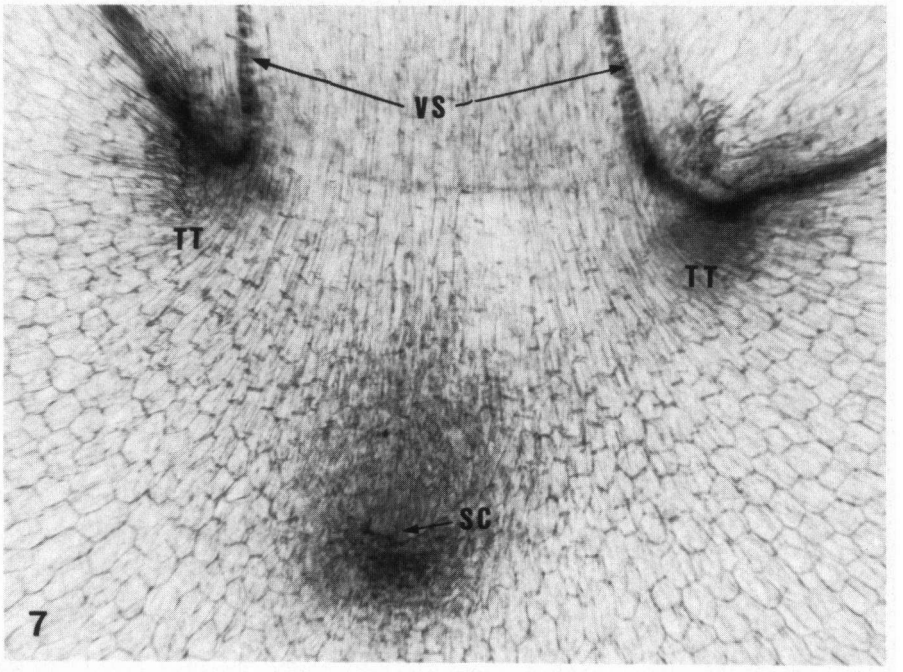


Fig. 6. Upper ovary viewed in profile, whole mount. St, stigma; SC, entry of the so-called stylar canal, which has no function in conducting pollen tubes; VS, one of the two vascular strands entering from the stigma; the other passes into the wall on the far side of the ovary. The transmitting tract departs from the vascular strand just above the level of the arrow to pass through the ovary wall. × c. 110.

of the ovary observed in such a section will be taken here as a line between the zone of insertion of the stigma and the centre of the basal vasculation, and tissues on the rachis side of this line will be referred to as adaxial and those on the other as abaxial.

The upper ovary is seen in profile in the micrograph of *fig. 6*. The stigma joins at an angle, inclined towards the adaxial face. Abaxially to the point of insertion lies the opening of the stylar canal (GUIGNARD 1901), which penetrates through the wall but plays no part in the conduction of the pollen tubes. The two vascular strands from the stigma enter the upper wall and immediately diverge to pursue pathways on opposite sides of the ovary to the base, where they unite with the main vasculation of the pistil. The two pollen-tube transmitting tracts, closely apposed to the vascular strands in the stigma (Y. HESLOP-HARRISON *et al.* 1984a), lose this association at the point of junction with the ovary wall, each passing directly through the wall independently of the vasculation. The architecture will be clear from *figs. 7 & 8*, which are of isolated upper ovary walls viewed from without and within.

Single transmitting tracts in the ovary wall are seen in radial longitudinal section in *fig. 9* and in transverse section in *fig. 10*. In the upper part of the wall the tissue of the two tracts differs in no essential respect from the corre-



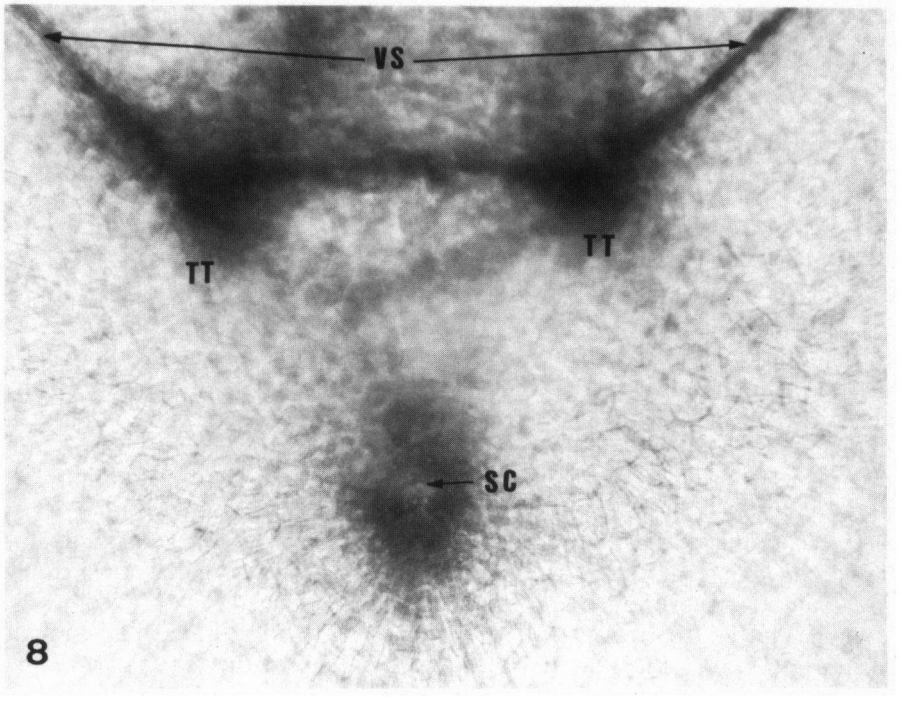


Fig. 7. Upper ovary viewed from above, showing the vascular strands (VS) entering from the stigma, and the associated transmitting tracts (TT). The entry of the stylar canal is seen at SC. × c. 140.
Fig. 8. As *fig. 7*, view from inside the ovary. × c. 140.

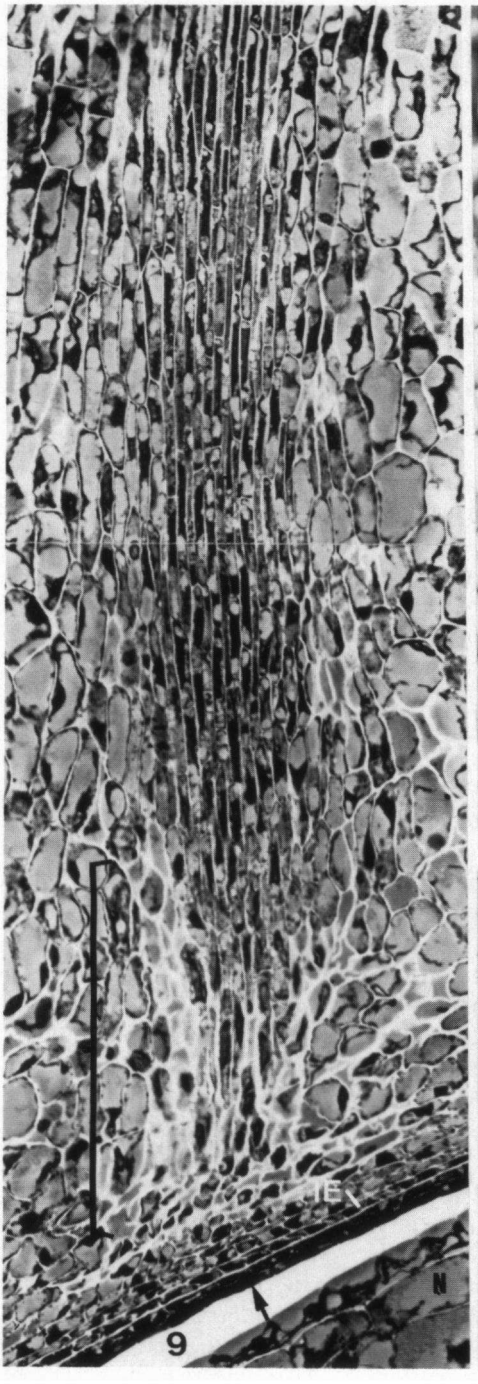
sponding tissue in the stigma. The columns are each composed of ranks of elongated, fusiform cells, 70–100 in number as viewed in section. As *fig. 9* shows, however, these columns terminate below in a mass of tissue made up of a core of elongated cells surrounded by a sheath of less regular cells distinguished by the possession of thicker walls. Sections through the region show that the number of ranks of the characteristic fusiform cells is severely reduced at this point, to some 16–20 as seen in section. At the foot of each tract, these abut upon three of four cell layers flattened in the plane of the inner ovary wall, within the inner epidermis.

The epidermis itself is seen in facial view in *fig. 11*. Closely appressed to it lies the inner integument, as may be seen at the arrow in *fig. 9*. The outer integument extends over the opening of the stylar canal, usually pushing slightly into it as noted by WEATHERWAX (1917) and RANDOLPH (1936), but it stops short of the two sites where the columns of transmitting tissue terminate; the inner integument therefore forms the only tissue between the inner ovary wall and the nucellus at these sites.

Because of the fact that pollen tubes are constrained to pass between the inner epidermis of the ovary wall and the inner integument in their passage to the micropyle, the conformation of the apposed cell layers is of some significance. The cells of the inner epidermis are somewhat elongated, and radiate outwards from a focus near the exit of the stylar canal. This orientation is preserved over most of the surface abutting the inner integument – that is to say, over that part of the adaxial face left uncovered by the outer integument. The disposition of the epidermal cells in the equatorial region of the ovary is seen in *fig. 12*, in which the orientation of the underlying hypodermal cell layers – at right angles to those of the epidermis – may also be distinguished. The cells of the closely appressed inner integument are also elongated in this region, and their orientation roughly matches that of the cells of the epidermis of the wall. In contrast, the epidermal cells of the nucellus show little indication of any preferred orientation in the upper ovary, although some polarisation in the longitudinal plane is evident at the equator.

The inner ovary wall, inner integument and outer nucellar cells are seen in section in *fig. 13*. This micrograph shows these tissues on the adaxial side of the ovule as observed in the longitudinal plane, the locus being approximately midway between the entry points of the transmitting tracts on the upper ovary wall and the micropyle. The cells of the inner epidermis, elongated in the plane of the section and therefore, as we have seen, along the longitudinal axis of the ovary, are crossed at right angles by the hypodermal cell layers (cf. *fig. 12*). The inner integument is composed of two cell layers, also elongated longitudinally in conformity. The epidermal layer of the nucellus is differentiated from the subadjacent layers, the cells being smaller and less vacuolate.

Fig. 14 shows the distribution of cutinised surface in a tissue section comparable with that of *fig. 13*. The cuticle of the nucellar epidermis is well defined and continuous, matching the inner cuticle of the inner integument, which in this section is drawn somewhat away from it. In sharp contrast the cuticle of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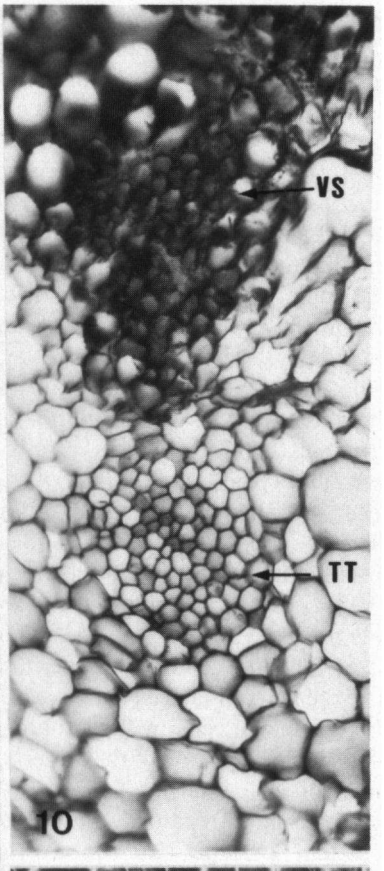
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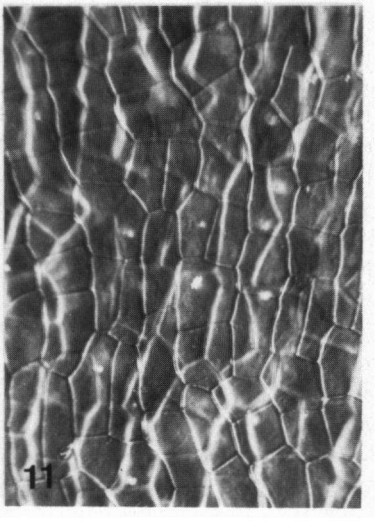
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Fig. 12. Facial view of the epidermis of the inner ovary wall in the equatorial region. Whole mount, DIC. The arrow indicates the direction of the micropyle. The epidermal cells are seen to be elongated in this site, with the long axis oriented longitudinally – that is, in the plane of the arrow. The hypodermal cell layers, seen through the epidermal cells in this micrograph, are oriented at right angles. × c. 250.

the epidermis of the inner ovary wall and the outer cuticle of the inner integument, so closely apposed in this area as to appear as a single entity, are extremely irregular and show occasional discontinuities. *Fig. 15* is of a stretch of ovary wall, inner integument and nucellus from the same preparation as that in *fig. 14*, but nearer to the termination points of the transmitting tracts of the upper ovary wall. The nucellar cuticle and the inner cuticle of the inner integument are here in close contact and are not separately resolved, but the fact that they are very well defined and continuous is clear enough. Again in contrast, the cuticularisation of the apposed cell layers of the inner ovary wall and inner integument is obviously quite tenuous, and indeed not resolvable at all over considerable stretches.

Fig. 9. Median longitudinal section of one transmitting tract in the upper ovary wall. Semi-thin (1.5–2 μm) section, resin embedment, Coomassie blue G staining. The upper part of the tract consists of elongated fusiform cells, 70–100 in number viewed in transverse section, much as in the transmitting tracts of the stigma. The number of elongated core cells diminishes below, where the tract is enclosed in a collar of irregular thick-walled cells (bracketed zone). The tract terminates in 3–4 layers of cells flattened in the plane of the inner epidermis (IE). The arrow points to the inner integument. N, nucellus. × c. 400.

Fig. 10. Upper ovary wall, freeze-sectioned in a tangential plane to show the separation of one vascular strand (VS) from the associated transmitting tract (TT). × c. 550.

Fig. 11. Facial view of the epidermis of the inner ovary wall at the the foot of one of the transmitting tracts; whole mount, DIC. There is no evidence of any special differentiation of the cells in this site to allow for the passage of pollen tubes. × c. 800.

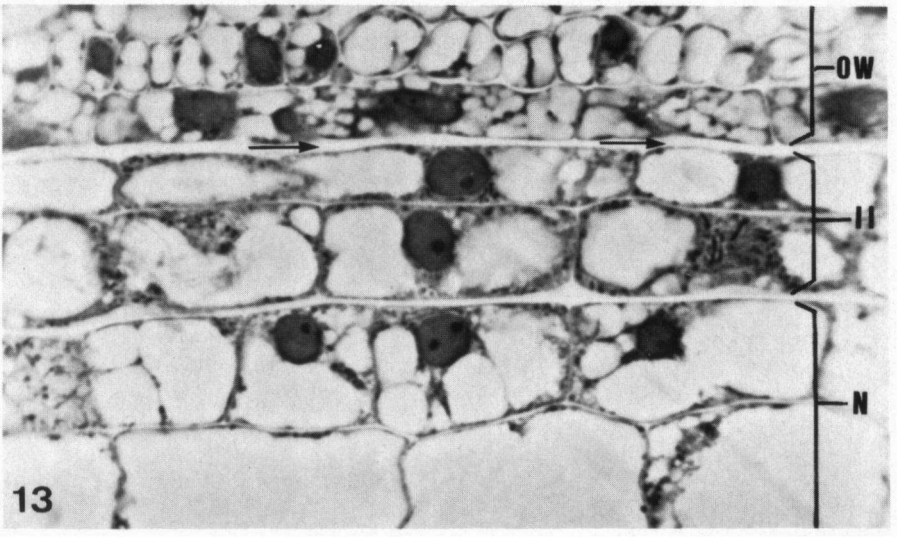


Fig. 13. Semi-thin section (1.5–2.0 μm) of the inner ovary wall (OW), inner integument (II) and nucellus (N) cut in the longitudinal plane in a site approximately midway between the entry points of the transmitting tracts and the micropyle on the adaxial side of the ovule. Resin embedment, Coomassie blue G staining. The arrows show the gap between the inner ovary wall and the inner integument which forms the pathway for the pollen tubes. The epidermal cells of the wall, both cell layers of the integument and the epidermal cells of the nucellus all have some characteristics suggestive of a secretory function, including abundant organelle populations, but no secretion was preserved in the intervening spaces by the present preparation methods. × c. 1500.

3.3. The micropylar region

The micropyle, integuments and inner ovary wall are seen in longitudinal section in *fig. 16* (inverted in relation to *fig. 6* to bring the embryo sac into a more familiar orientation, with the synergids towards the top of the illustration). The nucellar cells are disposed in parallel radially-oriented columns in this region. Four to eight of these columns of cells form a prominence that extends into the micropyle, the outermost cells being conspicuously larger than those of the rest of the nucellus. *Fig. 17* shows that these cells have virtually no cuticle. The facing epidermis of the inner ovary wall bears an irregular and discontinuous cuticle here as elsewhere (cf. *figs. 14 & 15*), and the cuticles of the epidermes of the adjacent inner and outer integuments are similarly thin and broken.

3.4. The distribution of secretions and secretory tissues

As in the corresponding tissues of the stigma, the intercellular spaces of the transmitting tracts in the upper part of the upper ovary wall contain a secretion product staining for protein and pectic polysaccharide, but no intercellular secretion was observed in the thick-walled, irregular tissues encasing the reduced tracts near their termination.

Nor could protein or polyssaccharide secretion be detected between the inner

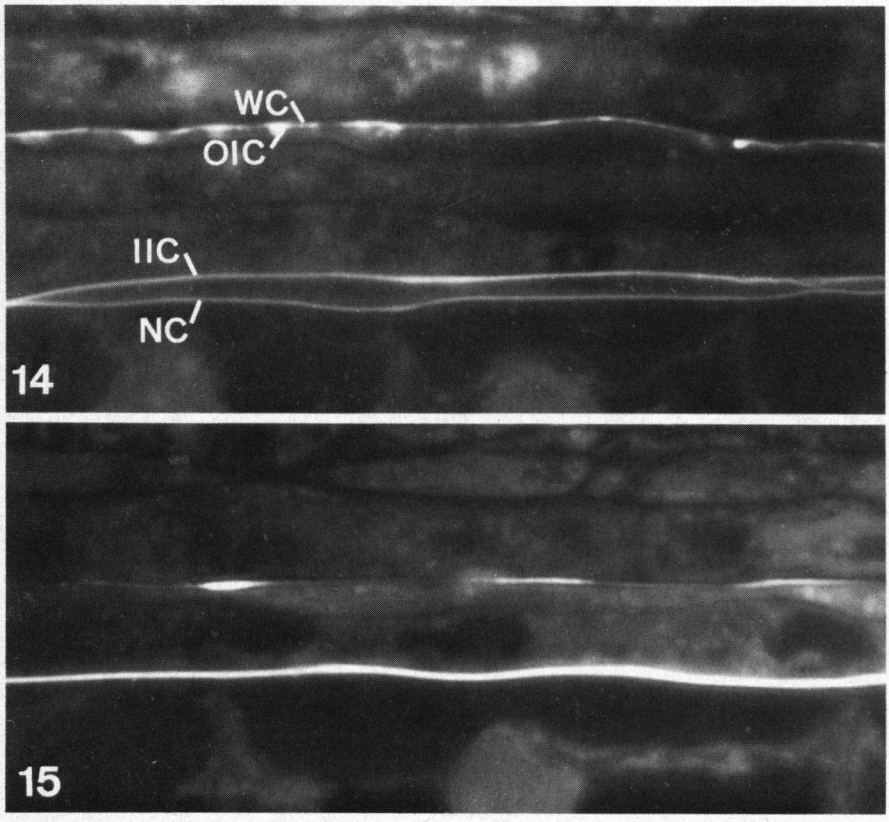


Fig. 14. As *fig. 13,* fluorescence micrograph, auramine O staining for cutin. The cuticles of the nucellar epidermis (NC) and the inner epidermis of the inner integument (IIC) are well defined and continous, but those of the wall epidermis (WC) and outer epidermis of the inner integment (OIC) are closely apposed, and are tenuous and in places discontinuous. × c. 1500.
Fig. 15. As fig. 14, site nearer to the termination points of the transmitting tracts in the upper ovary wall. × c. 1500.

integument and the inner ovary wall in the unpollinated but receptive ovary, although the structure of the cells of the adjacent tissues suggests a glandular function (*fig. 13*), as do the corresponding tissues in the ovary of *Hordeum vulgare* well illustrated by NORSTOG (1974).

The fixation and preparation procedures used in the present study preserved very little of the polysaccharide secretions in the vicinity of the micropyle so graphically demonstrated in *Paspalum* by CHAO (1971, 1977) by the use of freeze-substitution and non-aqueous processing. Copious alcian-blue staining mucilage was observed in this part of the ovule in fresh dissections of receptive but unpollinated ovaries of *Zea,* but this material, which no doubt must represent the secretion localised by CHAO, was evidently mostly lost during processing through aqueous media. The standard procedures did, however, preserve a con-

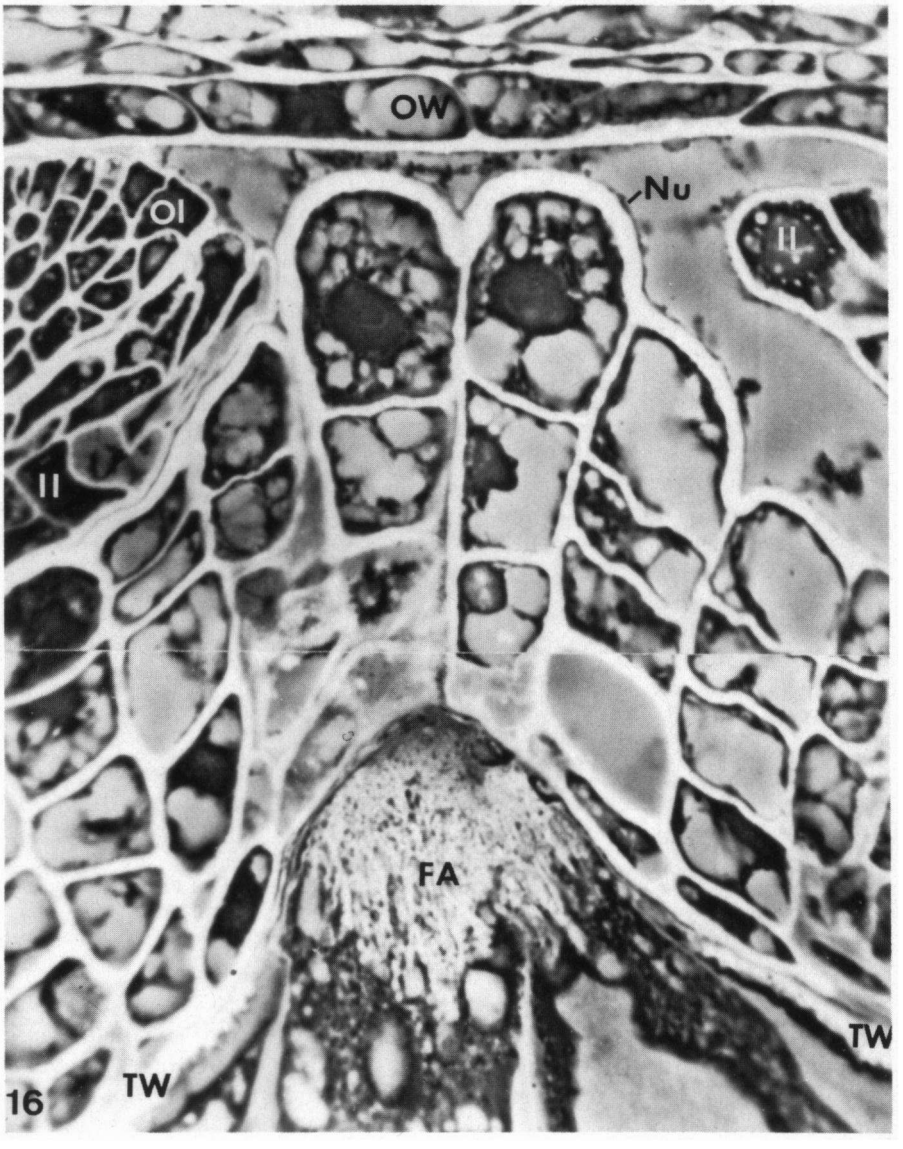


Fig. 16. Semi-thin section (1.5–2 μm) of the micropylar region of an unpollinated but mature ovule, cut in a median longitudinal plane, inverted in relation to *fig. 6*. Resin embedment, Coomassie blue G staining. OW, ovary wall; OI, outer integument; II, inner integument; Nu, nucellus; FA, filiform apparatus of the synergids; TW, transfer-cell type walls of the central cell of the embryo sac lateral to the synergids. The cells of both integuments, the ovary wall and the outer cells of the nucellus all have features suggestive of a secretory function. Protein is present in dense condensates in the space above the nucellus and between the integuments and the wall, but the preparation procedure has not conserved polysaccharide secretions in these sites. × c. 1900.

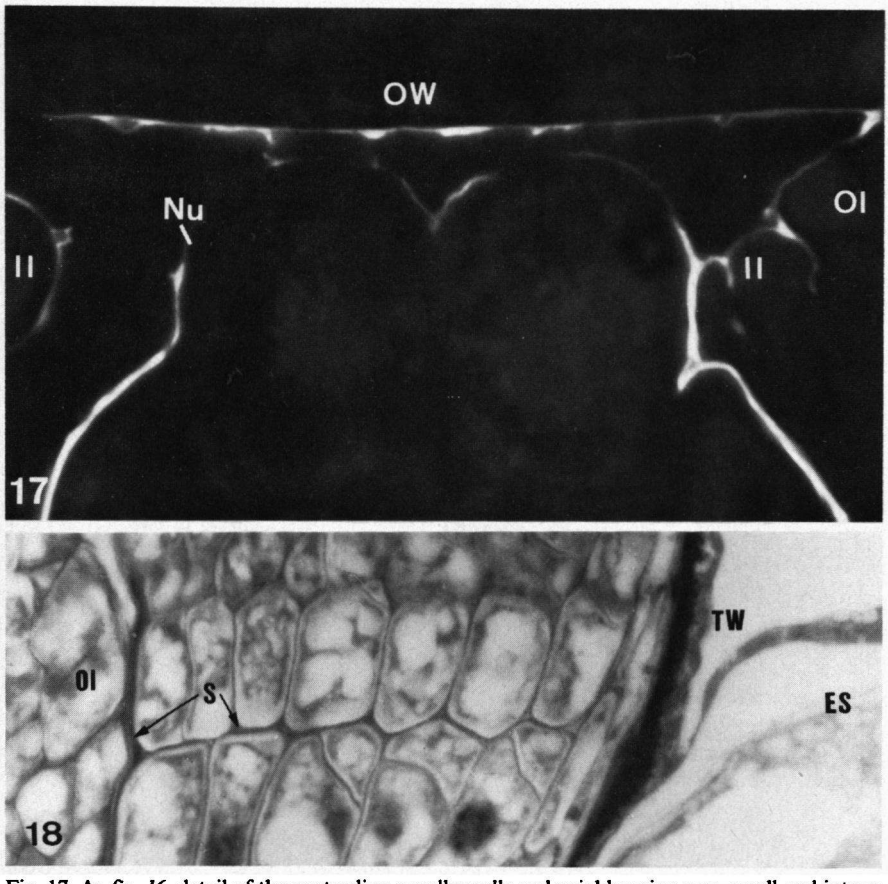
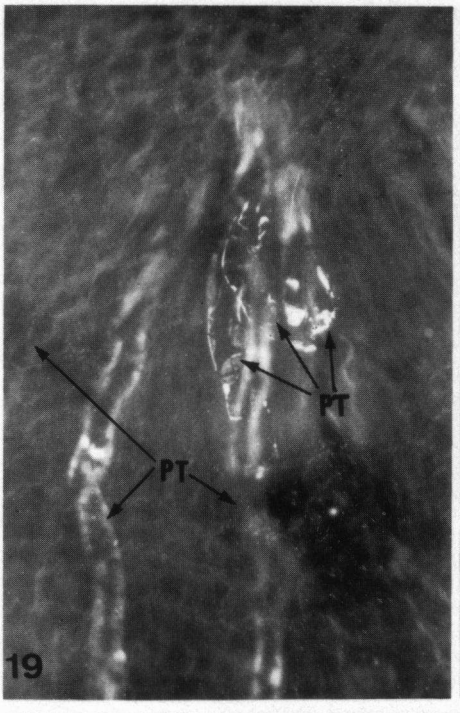


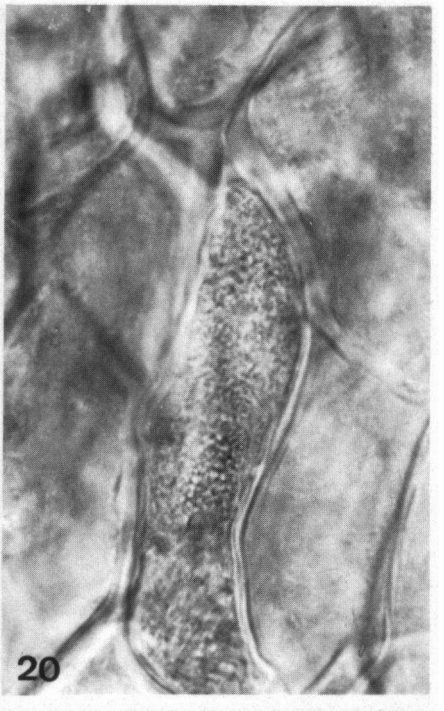
Fig. 17. As *fig. 16*, detail of the protruding nucellar cells and neighbouring ovary wall and integuments; fluorescence micrograph, auramine O staining for cutin. The exserted nucellar cells have a very thin and discontinuous cuticle, as do the adjacent cells of the wall and the integuments. × c. 2600.

Fig. 18. Detail of the intercellular spaces of the nucellus in the micropylar region of the ovule 6 h after pollination. Semi-thin (1.5–2.0 μm) section, resin embedment, alcian blue staining. At this time, the intercellular spaces are rich in pectic polysaccharide (S), like those of the transmitting tracts. Note that the transfer-cell type walls (TW) of the central cell of the embryo sac (ES) stain intensely with alcian blue (cf. *fig. 16*, where the staining is for protein). OI, outer integument. × c. 1800.

siderable amount of protein in the cavity around the micropyle, seen, for example, in *fig. 13* in the form of heavy condensates scattered over the protruding cells of the nucellus and between the integuments and the ovary wall. It is noteworthy that CHAO (1971) indicated that the secretion preserved by his methods at the micropyle of *Paspalum* may also have contained protein.

In unpollinated ovaries, pectic polysaccharide could be detected cytochemically in the intercellular spaces of the nucellar prominence, and this intercellular secretion was enhanced following pollination (*fig. 18*).





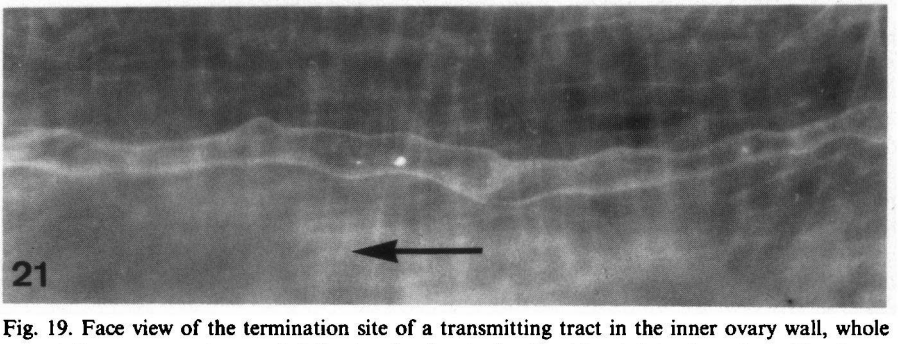


Fig. 19. Face view of the termination site of a transmitting tract in the inner ovary wall, whole mount, fluorescence micrograph following decolourised aniline blue staining for callose. The dissection was made 24 h after pollination. Six pollen tubes (PT) can be distinguished emerging through the inner epidermis and splaying out over the inner wall. The tubes show a general orientation towards the adaxial face, the most direct route over the inner integument to the micropyle. × c. 400.

Fig. 20. As *fig. 19*, whole mount, phase contrast, showing the passage of a single pollen tube into the ovary cavity, having broken through the tenuous cuticle (cf. *fig. 15*) between two epidermal cells. × c. 2500.

Fig. 21. Face view of the inner epidermis of the ovary wall in an equatorial site. Fluorescence micrograph, decolourised aniline blue staining for callose. The nucellus and inner integument were dissected away to reveal the tubes on the inner wall. The micrograph shows a single tube which has pursued a pathway almost directly towards the micropyle (direction indicated by the arrow). The tube has more or less followed the orientation of the walls of the epidermal cells, which in this site are oriented longitudinally (cf. *fig. 12*), with the hypodermal cell layers at right angles. × c. 500.

3.4. The pollen-tube pathway

Pollen tubes successful in passing the abscission zone at the stigma base enter the columns of transmitting tissue in the upper ovary wall, and grow thence without special hindrance until they reach the thick-walled tissue at the base of each column, where their progress is much more irregular. The severe reduction of the cross-sectional area of the tract evidently imposes competition, and this is another site where tubes may be displaced or arrested. Tubes that do pass the constriction grow through the intercellular spaces of the hypodermal layers of flattened cells, and then penetrate the thin cuticle of the inner epidermis and come into immediate contact with the inner integument. In our experience, they never penetrate the integument, but splay out immediately they make contact with it to push their way between the integument and the ovary wall. Thereafter their growth is mostly oriented towards the micropylar side of the ovule and away from the stylar canal and the overlapping outer integument. This polarisation is seen in *fig. 19*, which shows several entering tubes as seen from within the ovary. A detail of a single tube passing through the cuticle of the inner ovary wall appears in *fig. 20*.

The subsequent pathway of the tubes between the inner ovary wall and the integument is somewhat erratic, but in general they tend to follow the orientation of the outer, elongated cells of the ovary wall and the adjacent inner integument with occasional stepwise traverses across the cell files, as may be seen in *fig. 21*. MILLER (1919) recorded that the pollen tube '..twists and coils in its passage along the coats of the ovule until it reaches the micropyle'. We have not observed coiling in the ovary cavity, and extending tubes appear rarely to double back once a direction of growth has been established in the upper part of the ovary.

The shortest route over the surface of the inner integument between the entry point of the tubes and the micropyle in a receptive ovule of the variety used in this study is *c.* 1.8 mm. Assuming the tube attains a growth rate in the ovule comparable with that measured in the stigma, this distance would be traversed in 15–20 min. Entry into the sac is effected by the penetration of the successful tube though the intercellular spaces between the columns of protruding nucellar cells, spaces that now contain abundant secretion product (*fig. 18*).

4. DISCUSSION

The pollen tube pathway from the transmitting tracts of the stigma to the micropyle is made up of defined channels interrupted by obstacles of greater or lesser significance. The first of the latter, and probably the most effective in limiting pollen tube ingress into the ovary, is that at the site of the abscission layer at the stigma base. This presents no barrier to the first group of tubes entering from the stigma, but thereafter the rapid degeneration of the tissues at the abscission zone effectively obstructs the passage of further tubes. These changes in the abscission zone form part of a number of post-pollination effects in the ovary that appear to be activated by the presence of tubes in the upper wall. The conse-

quence of the blockage is, of course, to restrict the number of tubes to the minimum required to initiate the post-pollination events, whatever the number actually present in the stigma distal to the abscission zone.

Pollen tubes that do penetrate into the upper ovary wall have no difficulty in traversing the upper transmitting tracts, where guidance is undoubtedly given by the architecture of the tissue, as in the stigma itself. Thereafter the tubes meet two further potential obstacles – the constriction of the tracts before the inner wall is reached, and the cuticle of the inner epidermis. The considerable reduction of the cross-sectional area of transmitting tissue at the termini of the tracts must inevitably restrict the number of tubes reaching the inner epidermis still further, and it is to this that the loss of orientation that tubes often show in the upper ovary wall can no doubt be attributed. On the other hand, the cuticle itself is not a particularly effective barrier. As we have seen, it is thin and discontinuous, and penetrating it may not require enzymic dissolution (*fig. 20*).

The whole of the interface between the inner ovary wall and the inner integument on the adaxial side of the ovary would appear to be available for the further advance of the tubes once they have entered. In their passage, the tubes tend to follow the orientation of the inner epidermal cells and the cells of the contiguous integument. Transgressions do occur, and also some random growth, but in the main directionality is maintained, a fact which suggests that the surfaces between which they are growing provide a degree of guidance. The apposed cuticles are thin and discontinuous, and it therefore seems improbable that the tubes follow cuticular ridges. It is more likely that the conformation of the longitudinally oriented walls itself provides the directional cue (*figs. 12 & 21*).

Whatever part mechanical guidance might play in earlier sectors of the pollen tube pathway, no structural features of the tissues in the vicinity of the micropyle itself would appear to be adapted for such a function. The tube seeks the nucellus, and having penetrated, it grows more or less directly towards the filiform apparatus of the embryo sac. The site of entry is normally at or near the uncuticularised tip of the multicellular prominence (*figs. 16 & 17*), the tube entering directly into the intercellular spaces. The behaviour is comparable with that shown by the tubes in seeking the transmitting tracts at the beginning of their passage through the stigma, and once again the most plausible interpretation is that chemotropic guidance is involved.

The general conclusion to be drawn from the present study and earlier investigations of the *Zea* stigma (Y. HESLOP-HARRISON *et al.* 1984a & b) is that the passage of pollen tubes is a strictly regulated process. Provision exists for pollen adhesion and hydration, for tube guidance and nutrition, and for the progressive reduction of the numbers of tubes from whatever indefinite number may originally be captured to the one required for the double fertilisation. The findings do suggest ways in which the system may be affected experimentally, for example to facilitate the passage of alien pollen tubes for the purpose of hybridisation. However, it has to be acknowledged that our understanding of the physiology of many aspects of the interaction – for example, of the presumed chemotropic controls and the post-pollination effects – remain quite inadequate, and this is likely to be a limiting factor in attempts to manipulate the system.

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