# LOCATION OF THE POSTGENITAL FUSION IN THE GYNOECIUM OF CAPSELLA BURSA-PASTORIS (L.) MED.

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SUMMARY

Some methods, based on the characteristics of the epidermal outer cell wall, for finding the exact location of a postgenital fusion were tried on the false septum in the gynoecium of *Capsella bursa-pastoris* (L.) Med. Electron microscopical demonstration of the remnants of the enclosed cuticles proved a reliable means for locating the suture at the place of fusion in the septum.

## 1. INTRODUCTION

Postgenital (= ontogenetic) fusion of flower parts occurs in several families of higher plants (e.g. BAUM 1948a). The course of fusion does not vary much: the parts are forced together by growth, the epidermal cells interlock and the enclosed cuticle, if present, disappears. In the fused epidermal layers divisions, mostly periclinal, can occur that disturb the original cell pattern and so obscure the exact place of fusion. When this happens early in ontogeny, the postgenital fusion is easily overlooked or misinterpreted as a congenital (= phylogenetic) union, when farther developed stages are examined (MOELIONO 1970).

The object of this paper is to locate exactly the position of the suture between postgenitally fused parts in all stages of development by studying the characteristics of the epidermis. An epidermal outer cell wall differs from other cell walls in the following points: it is usually coated with a cuticle, it lacks a middle lamella, it has no plasmodesmata, it may have ectodesmata. If any one of these characteristics is preserved during fusion, it could indicate the exact place of the suture. For this introductory study a well known and easily localizable postgenital fusion was selected, namely, that in the false septum in the gynoecium of *Cruciferae*. Young gynoecia with incompletely fused septa can be found easily, while in a full-grown septum no suture can be demonstrated (HANNIG 1901). It is therefore a suitable object for studying the full course of fusion.

## 2. MATERIAL AND METHODS

Inflorescences of *Capsella bursa-pastoris* (L.)Med. were collected along the roadside.

Material for light microscopy was fixed in a mixture of formalin, propionic acid and ethanol, dehydrated with tertiary butyl alcohol, and embedded in

Paraplast. Serial sections of 7 μm, transverse and longitudinal, were stained with astra blue and safranin, some with Sudan III or ruthenium red.

For electron microscopy gynoecia were isolated and the valves and the ovules were removed to promote access of the fixative to the septum. The material was fixed at room temperature in unbuffered 2% $OsO_4$ for 1–15 hrs or in 4% glutaraldehyde in 0.02 M phosphate buffer (pH 7.3) for 3 hrs, followed by 2% $OsO_4$ in the same buffer for 1–2 hrs. In some cases fixation was done in unbuffered 2% $KMnO_4$ for 5–15 min, with satisfactory results (not shown). After dehydration with a graded ethanol series the material was embedded in Epon 812, or in n-butyl methacrylate: styrene = 7:3 (MOHR & COCKING 1968). Sections were made with glass knives on an LKB Ultrotome, mostly poststained with lead citrate (REYNOLDS 1963) and examined in a Philips EM 100B. Only transverse sections through the middle or near the top of the gynoecium were used.

## 3. RESULTS

### 3.1. Light microscopy

#### 3.1.1. Ontogeny of the septum

The gynoecium of the *Cruciferae* is supposed to be composed of two congenitally united carpels. The septum in it is formed by two outgrowths of the carpels along their postulated line of union. The outgrowths become appressed to each other acropetally in the middle of the gynoecial cavity and fuse with their margins (*fig. 1*). During subsequent extension growth the greater part of the septal mesophyll breaks down; in *Capsella* only the fused cells and some cells adjoining them survive. They constitute a cell bridge between the septal epidermises that is conspicuous in transverse sections of the gynoecium. Location of the place of fusion is facilitated by this (*fig. 2, 3*). The cell walls in the bridge thicken considerably so that original cell form and arrangement are obscured.

#### 3.1.2. Staining reactions

Thickening of the cell walls in the bridge between the septal epidermises is due to the deposition of cellulose and some pectin or pectinous material, as shown by the staining with astra blue and ruthenium red, respectively. This agrees with the results of HANNIG (1901). Staining with astra blue is most intense just around the cells.

Ruthenium red staining makes visible a layer of pectin at the outside of the septal epidermis and an indistinct, fragmentary network of middle lamellae between the cells of the bridge, also at the probable place of fusion.

With Sudan III no trace of a cuticle can be demonstrated on the septal epidermis, nor in the cell bridge, at any stage of development.

### 3.2. Electron microscopy

#### 3.2.1. The cuticle

In all stages of development the septum is covered by a heavily stained layer with a thickness of 15–50 nm. This layer is present both with permanganate and

osmium tetroxide fixation. Considering its location and staining properties, the layer probably is the cuticle; it will be named so further on.

Before they touch, both halves that will form the septum have their own cuticle. Parts of these cuticles are enclosed during fusion, thus constituting a distinct suture in the septum because the enclosed cuticles keep their staining properties. When the septal halves have just become appressed, two cuticles can be discerned at the suture, lying close but not merging with each other; the space between the cuticles is unstained (*fig. 4*). In later stages of fusion the enclosed cuticles merge to form a single cuticular layer that connects the cuticles on the septal epidermises (*fig. 5*). This cuticular layer gradually breaks down, often beginning in places where the lumina of the fused cells are closest (*fig. 8*). In full-grown septa only few and small fragments mark the suture; staining of these fragments is reduced, least so at the edges, perhaps as a result of progressive polymerization of the cuticular substance. However, by their presence the suture can still be located without much difficulty (*fig. 9*).

### 3.2.2. Plasmodesmata

In young gynoecia there are numerous plasmodesmata in the anticlinal walls of the epidermal and fused cells, but as wall thickening proceeds, their number decreases. Between the fused cells no plasmodesmata are found, except occasionally in places where the cuticular layer is absent and secondary wall thickening is still negligible (*fig. 6*). In some instances seeming ectodesmata are seen running from the cell lumen to the cuticular layer (*fig. 7*). These are probably obliquely cut plasmodesmata; ectodesmata do not show with the fixatives used (MARTIN & JUNIPER 1970).

### 3.2.3. Middle lamella and cell wall

With the methods used no middle lamella can be discerned between the cells. The fused cell walls have the same appearance as the other cell walls in the septal bridge. Before thickening they look homogeneous, while in old stages, with pronounced thickening, the walls are two-layered. One layer with a clear striation, probably caused by cellulose microfibrils, surrounds each cell individually; the other layer, without a clear structure, lies between the cells, including the fused ones.

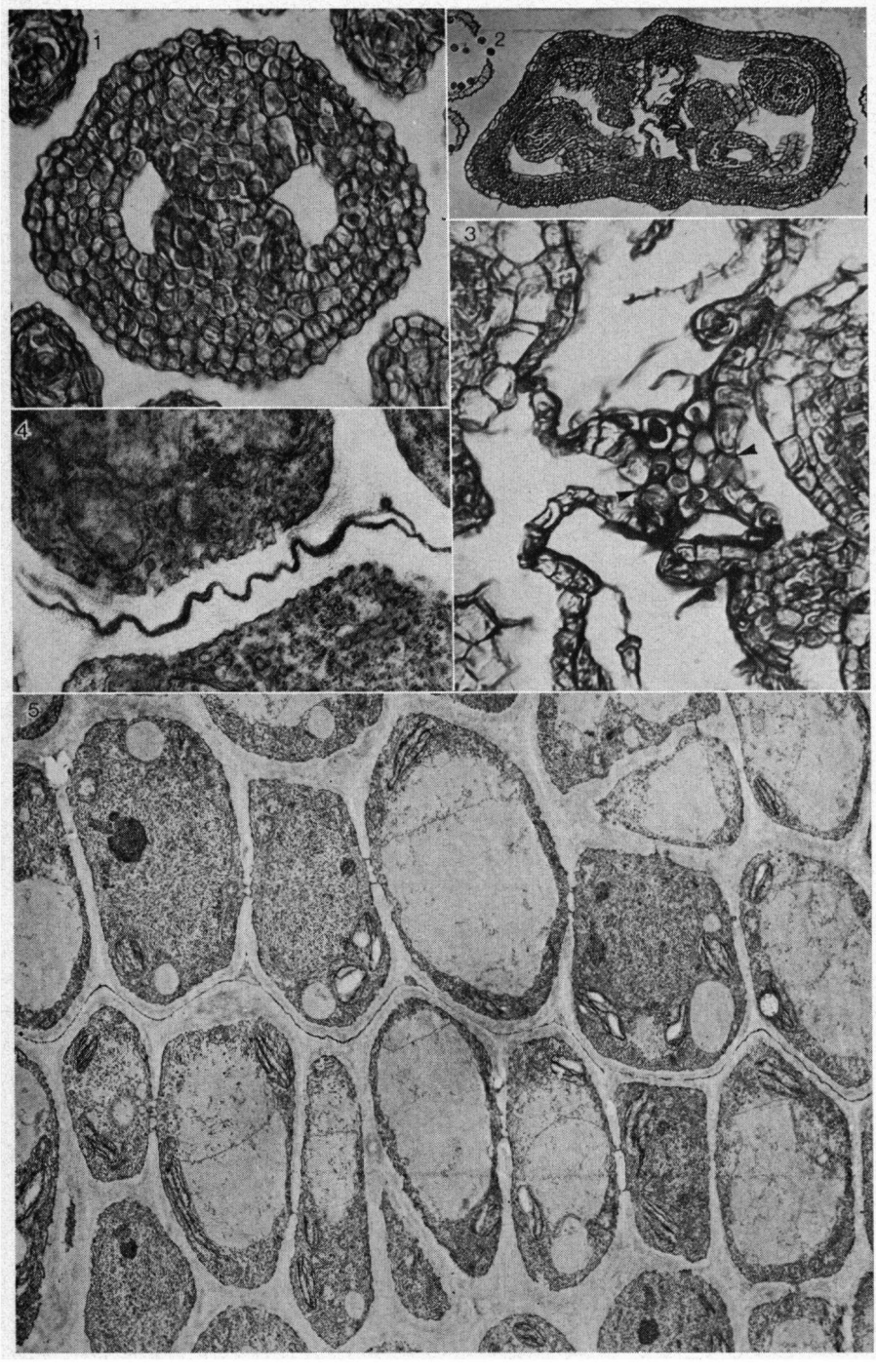
Fig. 1. Very young gynoecium, length (from the insertion of the stamens to the top of the pistil) 0.2 mm. Light micrograph of a transverse section through the middle, stained with astra blue and safranin. × 700.

Fig. 2. Gynoecium, just before anthesis, length 1.0 mm. Data as *fig. 1*. × 130.

Fig. 3. Septal cell bridge, detail of *fig. 2*. The probable place of fusion is indicated by arrows × 700.

Fig. 4. Detail of the suture shortly after the septal halves have become appressed. The cuticles are still separate. Length of gynoecium 0.4 mm, transverse section near the top. Fix. $OsO_4$ 1 hr, embedded in methacrylate-styrene, stained with lead citrate. × 22000.

Fig. 5. Suture in the septum, marked by a nearly unbroken cuticular layer. Transverse section through the middle of a gynoecium, 0.5 mm long. Fix. $OsO_4$ 1 hr. × 4700.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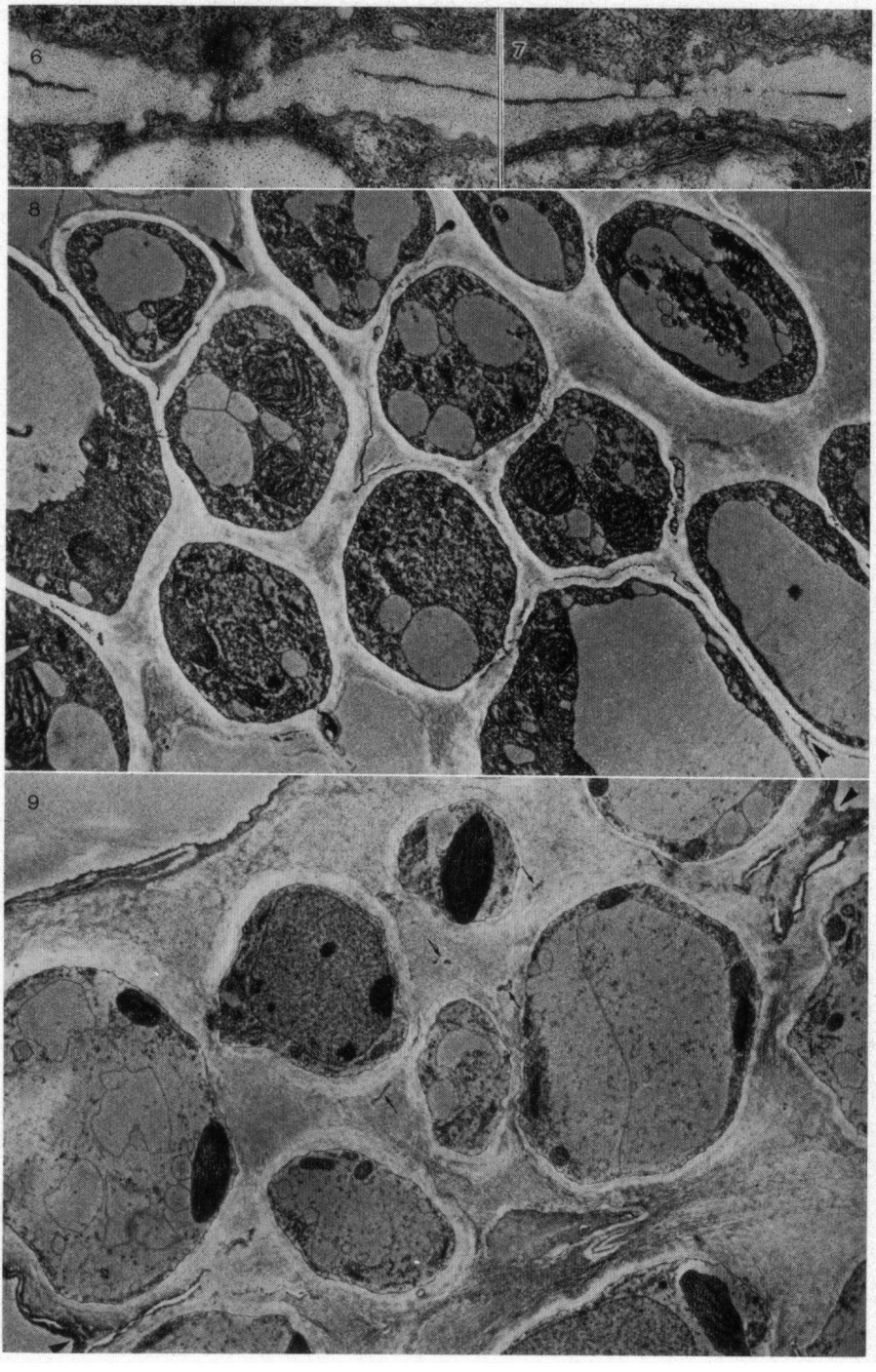
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## 4. DISCUSSION

The remains of the enclosed cuticles prove to be distinct marks for the location of the suture in the postgenitally fused septum in the gynoecium of *Capsella*, in all stages of development. The cuticles are too thin to be seen with the light microscope.

Another means for locating the suture is the scarcity of plasmodesmata in the fused cell walls, as compared with the other walls of the fused cells. However, during wall thickening nearly all plasmodesmata disappear, so this means can only be used in early stages of fusion. The occurrence of plasmodesmata between the fused cells seems to be restricted to a brief stage of development.

With the methods used it is not clear whether the appressed cells fuse sutureless in those places where the cuticular layer has disappeared. Possibly some irregularity in the deposition of cellulose or pectin can be demonstrated, thus constituting a third means for locating the suture.

It is not known what causes the breakdown of the enclosed cuticles, though there are some arguments in favour of growth as the disruptive factor. In the first place, from the moment when the septal halves become appressed to the moment when the growth of the septum ceases, increase in length of the fused parts is at least tenfold, increase in width about twofold. No example is known of cutin deposition in a postgenital fusion, except in intercellular spaces (BAUM 1948b), so probably the cuticular substance that is enclosed at the suture has to be spread over a considerably greater area during growth of the septum. This could easily cause rupture of the cuticular layer, the more so as the initially soft and flexible cuticular substance tends to harden by progressive polymerization.

In the second place, breakdown of the cuticular layer is seen to give rise to many fragments of varying sizes, apart from each other but with about the same thickness as the intact cuticular layer. The larger fragments are found in places of relative rest during growth, as at corners between cells. With enzymic digestion a gradual and less local disappearance of the cuticular layer would be expected. At this stage, however, enzymic action should not be definitely discounted (CUSICK 1966).

Fig. 6. Plasmodesmata in fused cell walls. Transverse section through the middle of a gynoecium, 0.6 mm long. Fix. $OsO_4$ 15 hrs, stained with lead citrate. × 26000.

Fig. 7. Seeming ectodesmata in fusing cell wall. Data as *fig. 6*. × 26000.

Fig. 8. Suture in the septum, marked by large fragments of the cuticular layer. Both ends of the suture are indicated with arrows. Transverse section through the middle of a gynoecium, 1.7 mm long. Further data as in *fig. 4*. × 4700.

Fig. 9. Suture in the septum, marked by several small fragments of the cuticular layer (small arrows). Both ends of the suture are indicated with arrows. Transverse section through the middle of a gynoecium, 4.8 mm long (nearly maximal length). Fix. glut. ald. 3 hrs + $OsO_4$ 2 hrs, embedded in metbacrylate-styrene stained with lead citrate. × 4700.

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