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POSTGENITAL FUSION IN THE GYNOECIUM OF THE PERICLINAL CHIMERA LABURNOCYTISUS ADAMII (POIT.) SCHNEID. (PAPILIONACEAE)

J. H. BOEKE and G. J. C. M. VAN VLIET*

Biologisch Centrum, Rijksuniversiteit Groningen, Afd. Plantensystematiek, Haren (Gr.)

SUMMARY

The postgenital fusion of the carpel margins of the gynoecium of *Laburnocytisus adamii* (Poit.) Schneid. is described in detail using light and electron microscopy. In this species, the cells of dermal origin are characterized by osmiophilic bodies, facilitating the localisation of the enclosed dermal tissue at the site of fusion. The osmiophilic bodies are abundant in the outer epidermis and decrease in number and size towards the inner part of the enclosed dermal tissue. They are absent from the inner epidermis. Reasons for this distribution pattern are discussed. Variation in cell configuration and width of the enclosed dermal layer are probably caused by differences in growth rate between dermal and subdermal tissue.

1. INTRODUCTION

It is a well known phenomenon that meristematic plant parts, after making contact, can fuse to form one functional unit. It is less generally known that fusion of primordia is a normal event in the ontogeny of organs in many plant species. This unfamiliarity is understandable, since fusion often occurs very early in the ontogeny, and the signs of fusion usually disappear in older stages. This can be illustrated with the fusion of two primordia, where enclosed epidermal cells, normally differing in various aspects (e.g. shape, direction of division etc.) from the subdermal tissue, fully conform to the surrounding cells. The subdermal tissue then looks homogeneous.

Since BAUM (1948) published her observations on the course of postgenital fusions between carpel margins in a large number of plant species, it is known that such fusions all proceed in a similar way. The epidermal cells at the place of fusion react to enclosure with interlocking of opposing cells and periclinal divisions, and finally they fully conform to the surrounding subepidermal tissue. In the cell pattern that originates, the place of fusion can be nevertheless inferred by careful histogenetical analysis, even in advanced stages of fusion (BOEKE 1973b; MOELIONO 1970). Recently the process of fusion between carpel margins has been studied using transmission electron microscopy. It was found that remainders of the cuticle mark the fusion-boundary between two, originally free parts (LAMOND & VIETH 1972, MORRISON 1975, WALKER 1975a, b). Usually the enclosed cuticles disappear in later stages of development. After some time fused

* Present address: Hortus Botanicus, Plantage Middenlaan 2, Amsterdam

cell walls show all the characteristics of normal cell walls, even a pseudo-middle lamella and plasmodesmata (BOEKE 1971, 1973a, b). Such features can even be found in the walls between cells of different species, such as in periclinal species-chimeras (cf. BURGESS 1972). Finally, after the disappearance of the enclosed cuticles the place of fusion in normal plant tissue can only be located with histogenetical analysis (BOEKE 1973b) of the cell pattern. Periclinal chimeras, which have genetically different dermal and subdermal tissue form an exception to this rule. There are two main types of chimeras: species-chimeras, with components that originated from different species, and cytochimeras, with components that arose by partial polyploidisation of the apical meristem within a single plant (ploidy-chimeras) or which differ very little, for instance in one gen only (mutation-chimeras). The ontogeny of the flower parts in the cyto-chimera *Prunus persica* was studied comprehensively by DERMEN (1953, 1956) and DERMEN & STEWART (1973). They found that the place of fusion of the carpel margins was clearly marked by the difference in cell size between subdermal and the enclosed dermal tissue. This difference in cell size, related to the ploidy level of the cell was visible in advanced stages of development. The ontogeny of the fusion in this material was not treated in detail and so it seemed worthwhile to study this phenomenon more extensively.

The gynoecium of *Laburnocytisus adamii* (Poit.) Schneid., a well known ornamental tree, was chosen as a subject for this study. It is a periclinal species-chimera, composed of dermal tissue of *Cytisus purpureus* Scop. – a single layer wide at the meristem – surrounding core tissue of *Laburnum anagyroides* Med. Only the cells of *Cytisus* tissue contain dark staining bodies in their vacuoles. These so-called osmiophilic bodies are probably composed of phenolic substances (cf. BUDER 1911) and can easily be discerned with both light and transmission electron microscopy. Because of this feature, the components of this chimera can easily be separated from each other.

2. MATERIALS AND METHODS

Inflorescences of *Laburnocytisus adamii* (Poit.) Schneid. were collected in the Hortus Botanicus of the University of Amsterdam from the end of April through to June. Material was only taken from smooth skinned branches, not from hairy ones, as the latter are presumably reversions to pure *Laburnum anagyroides* Med., lacking the epidermis of *Cytisus purpureus* Scop. The gynoecia were isolated, opened lengthwise, fixed for 1 h in 2% unbuffered OsO_4 at room temperature, dehydrated in an ethanol series and embedded in Epon 812 via acetone. During dehydration the tissue was contrasted with uranyl acetate. Serial 1 μm sections, both transverse and tangential (perpendicular to the plane of fusion) were made with glass knives and stained with aqueous crystal violet at 70 °C; for details of the procedure see BOEKE (1973b). Ultrathin sections for electron microscopy were made with a diamond knife and examined in a Philips EM 100 and 201.

3. RESULTS

3.1. Ontogeny of the gynoecium

The gynoecium of *Laburnocytisus* is formed in the same way as the gynoecia of other Leguminosae studied (BOEKE 1973a, b). It is interpreted as a single carpel, folded lengthwise and with postgenitally fusing margins. The carpel primordium is horseshoe-shaped in transverse sections. The margins grow towards each other and touch, beginning at the base, when the carpel wall is ca. 1 mm long; at a length of ca. 2.5 mm, the enclosed cuticles have disappeared in some places and the fused cells are connected by plasmodesmata. The margins fuse last at the distal end of the carpel, thus closing the ovary cavity. The distal end of the carpel differentiates further into the solid style and the stigma. The oldest gynoecia that were studied, from flowers with withering corollas, had an ovary length of 15 mm and a style of 5 mm. In this stage the tissue between the ventral vascular bundles, i.e. at the place of fusion, already desintegrates from the inside outward, in preparation for the dehiscence of the ripe pod.

3.2. Distribution of osmiophilic bodies in the gynoecium

When the gynoecium has a length of ca. 1.5 mm and the carpel margins begin to fuse, some small osmiophilic bodies can be discerned by electron microscopy in the vacuoles (and cytoplasm) of the epidermal cells. These bodies were also observed by BURGESS (1972) and are probably identical with the phenolic or tannic bodies that were observed by BUDER (1911).

In older stages of development (*fig. 4*) the osmiophilic bodies are generally more numerous and of greater size. Sometimes the whole vacuole of a cell is occupied by a single giant osmiophilic body. The epidermal cells that are enclosed during fusion of the carpel margins, and their daughter cells all contain osmiophilic bodies, increasing in number and size during maturation. However, towards the interior of the gynoecium, the frequency of the osmiophilic bodies decreases, and they are absent from the epidermal cells lining the ovarial cavity (*fig. 2*).

3.3. Cell configuration at the place of fusion

The place of fusion of the carpel margins can be located in young stages by the "epidermal" cell shape in the two enclosed dermal layers, in old stages by the occurrence of osmiophilic bodies. The enclosed dermal tissue constitutes a layer that is generally two cells wide. The layer is situated between the ventral vascular bundles, and is connected to the inner and outer epidermis of the ovary wall. The contact places of the layer with the epidermides are marked by longitudinal furrows in the ovary wall. From the fact that the enclosed dermal layer is mostly two cells wide it can be concluded that the cell divisions in this layer are mainly perpendicular to the suture between the carpel margins. Occasionally the enclosed dermal layer was three cells wide; in these cases it could be inferred from the cell configuration that an enclosed dermal cell had divided parallel to the suture. Furthermore it was regularly observed that the enclosed dermal layer was

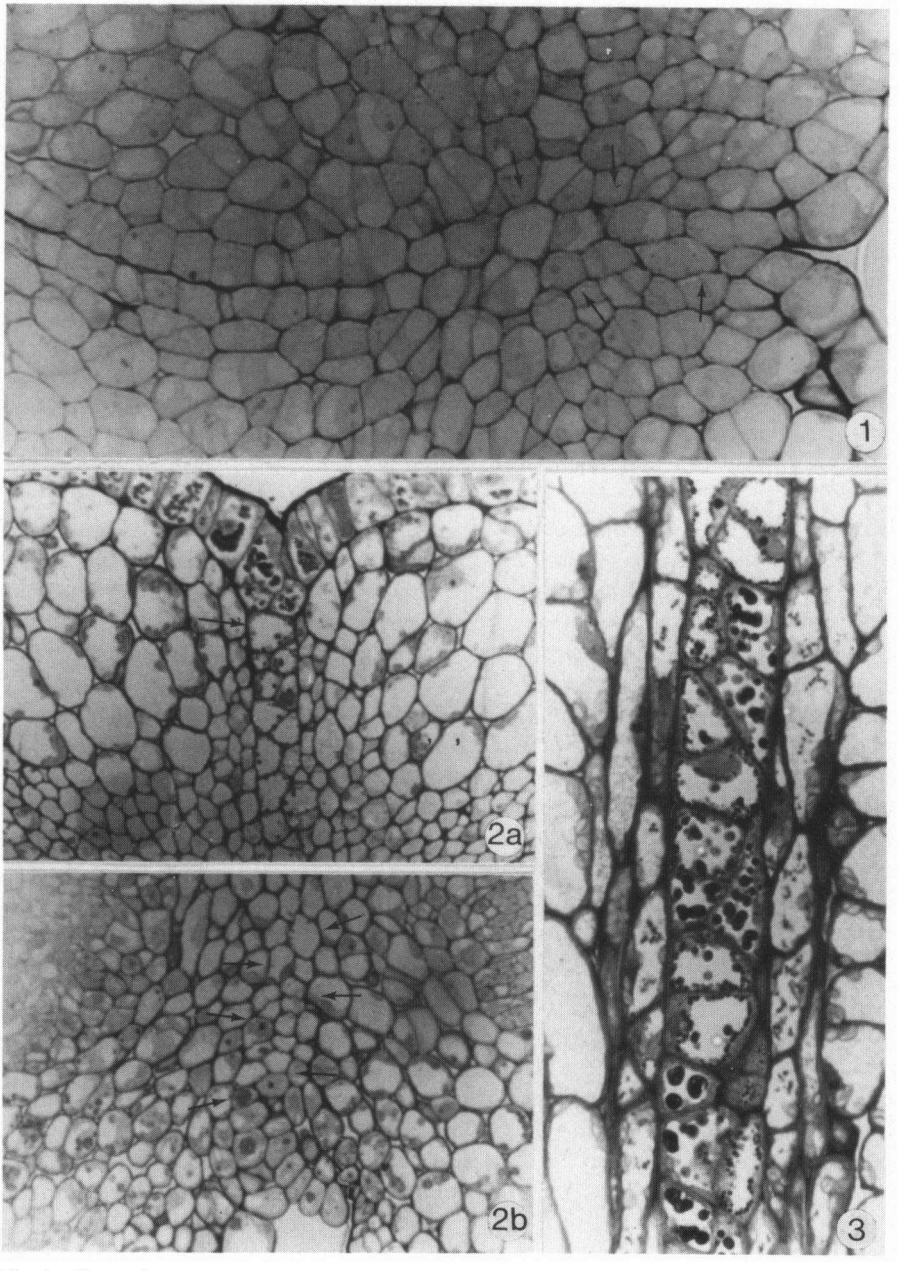

Fig. 1. Photomicrograph of a transverse section of the ovary wall in a very early phase of fusion. The epidermal cells in the inner part of the suture have not changed in shape yet, those on the more outer parts of the suture show various stages of interlocking. × 1200.

only one cell wide. An unexpected observation, since one would expect at least two cell layers, because the enclosed dermal layer is formed by two epidermal layers. This was only observed in older stages, close to the outer epidermis of the ovary wall. The plane of contact of the carpel margins increases considerably as a consequence of the subsequent growth in length and width (c. 15 and 5 times, respectively) of the ovary wall. The enclosed dermal layer keeps pace with this increase for, as mentioned above, it generally remains two cells wide and is not interrupted. In the enclosed dermal layer both cell elongation and anticlinal cell divisions occur. These processes probably account for the total increase in area of the enclosed dermal layer.

In early stages of fusion (length of the gynoecium ca. 3 mm) the epidermal cells are all alike, differing from the subdermal cells in shape and division pattern (*fig. 1*). During the extensive growth of the ovary wall, the enclosed dermal cells obtain different shapes (*fig. 2*), and in later stages of development (length of gynoecium 7 mm) the enclosed dermal cells near the outer epidermis are strongly interlocking and the suture between them has a marked zig-zag course. These cells are there arranged in longitudinal, spindle-shaped cell families. The subdermal tissue bordering this part of the enclosed dermal layer consists of cells that are clearly different, both in shape and arrangement, from the fused cells. Further towards the interior of the ovary wall the enclosed dermal cells are longitudinally more elongated, not arranged in spindle-shaped cell families, more rounded, while the suture between them is difficult to recognize. The difference in shape between the dermal and subdermal cells is not so well marked here (*fig. 2*).

In very old stages (ovary length c. 15 mm) the tissue between the ventral vascular bundles desintegrates, as mentioned before. The strongly interlocking enclosed dermal cells, near the outer epidermis, remain, however, intact presumably until dehiscence of the pod.

3.4. Electron microscopy of the suture

In early stages of fusion an uninterrupted cuticular layer can be observed in the middle of the enclosed dermal tissue (*fig. 5*). This cuticular layer, marking the suture between the carpel margins, is continuous with the cuticles of the outer and inner epidermis of the ovary wall. In gynoecia with a length over 2.5 mm, the

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Fig. 2. Photomicrograph of a transverse section of the ovary wall in a later stage of fusion than in fig. 1; the middle part of the section is omitted. × 950.

2a. Outer epidermis and outer part of the fused epidermis. Note the frequent occurrence of osmiophilic bodies and the occasionally one cell wide enclosed dermal layer (arrow); nearby cells are strongly interlocking.

2b. The dermal layer on the inner side of the suture (arrows) is always two cell layers wide and almost fully conforms to the subdermal layer.

Fig. 3. Photomicrograph of a tangential longitudinal section through the outer part of the ovary wall; the dermal cells are strongly interlocking and are arranged in spindle-shaped groups; osmiophilic bodies are of frequent occurrence. × 1000.

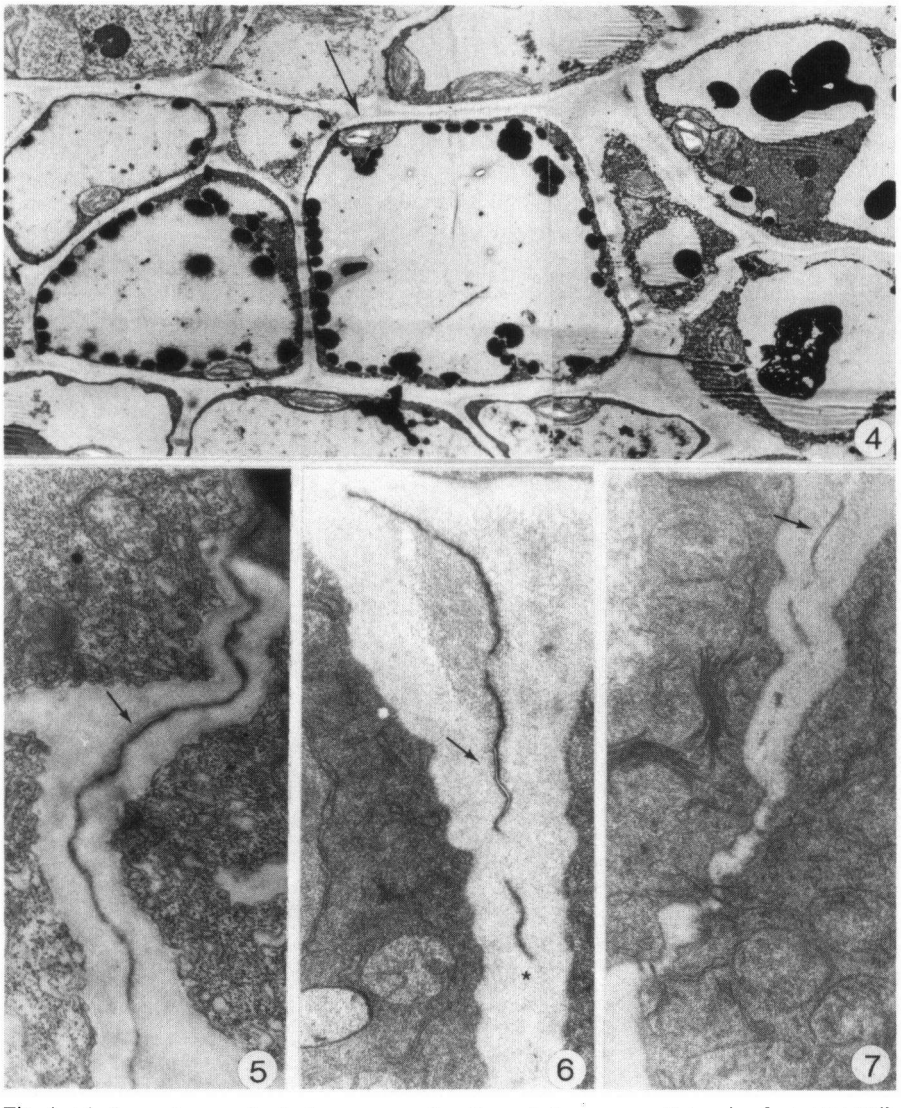

Fig. 4. Electron micrograph of a transverse section through the ovary wall showing frequent small and infrequent large osmiophilic bodies in the vacuoles of the enclosed dermal tissue, and the absence of those bodies in the subdermal cells. The single dermal cell in de middle of the micrograph (arrow) is identical to the one in fig. 2a that is marked with an arrow. ×4100.

Fig. 5–7. Electron micrographs of the suture between two epidermal layers. Note the infrequent occurrence of osmiophilic bodies in these early stages of fusion.

Fig. 5. The enclosed cuticular layer is still undisrupted, sometimes two layers can be recognized (arrow). × 17.000.

Fig. 6. Enclosed cuticular layers are disrupted, the original two layers can sometimes be recognized (arrow). × 19.000.

Fig. 7. Plasmodesmata are formed, the cuticle is even further fragmented, although the two original layers can still be recognized (arrow). × 14.000.

cuticular layer starts to break up into smaller fragments. At some places these fragments can still be seen to consist of two layers (*fig. 6*, arrows) as is consistent with their origin from the cuticles that covered both carpel margins before fusion. In even later stages of ovarial growth, the cuticles fall apart into even smaller, and more widely separated fragments. Finally it is no longer possible to locate the suture by remnants of the cuticular layer (length of the gynoecium c. 7 mm).

In places where the cuticular layer has disappeared, structures can be seen in the fused cell walls that closely resemble the plasmodesmata in the other cell walls (*fig. 7*). Most likely, these structures are really plasmodesmata. The electron microscopical observations mentioned above are in agreement with the results obtained in studies of other postgenital fusions (BOEKE 1971, 1973a, b).

4. DISCUSSION

Information in the literature on the behaviour of epidermal cells that are enclosed during tissue fusion of periclinal chimeras is scarce. Until now this process was only studied for *Prunus persica* (DERMEN 1953, DERMEN & STEWART 1973) and for *Datura* (SATINA & BLAKESLEE 1943). However, these were cyto-chimeras of which the cells of the tissues only differ in ploidy. Results from these studies are therefore difficult to compare with those presented in this paper, because the tissues of the species-chimera Laburnocytisus show a more distinct genetical difference.

The enclosed dermal layers of *Laburnocytisus* keep up with the radial and longitudinal growth of the ovary wall by means of cell division perpendicular to the suture and by cell stretching. Their contribution to cell formation in a tangential direction is only small, because periclinal cell divisions are infrequent. This pattern of cell division of the enclosed dermal tissue was also found in two other Leguminosae, *Trifolium* and *Lathyrus* (BOEKE 1973a, b).

The arrangement of enclosed dermal cells in longitudinal spindle-shaped cell families and the occurrence of one cell wide sites in the enclosed cell layer can be explained as a consequence of a different growth rate of dermal and subdermal tissue. BUDER (1911), studying the ontogeny of the ovulum of *Laburnocytisus*, observed that the nucellus protruded out of the micropyle in the course of its development; he attributed this phenomenon to inadequate growth of the integuments, which would not leave the nucellus enough room for its normal development, and he concluded that the growth rate of the dermal (*Cytisus*) tissue is slower than that of the non-dermal (*Laburnum*) tissue. This conclusion is supported by the fact that *Laburnum* is a fast-growing plant and that *Cytisus* has a more moderate growth rate. In *Laburnum anagyroides* the shapes of the enclosed dermal cells in a corresponding stage of development do not show an arrangement in spindle-shaped cell families, as was noticed during an exploratory examination.

The enclosed dermal cells at both sides of the suture have penetrated to various extents between each other by intrusive growth, and the tip of a spindle-shaped

cell family usually overlaps with the one above. Sometimes, however, they do not connect, leaving an interstice in the dermal layer at one side of the suture. At these places the opposite cell family in the dermal layer of the other side of the suture borders at two sides to the subdermal tissue, resulting there in an enclosed layer of only one cell wide. It is not evident why intrusive growth is only present near the outer epidermis, but it seems likely that this is caused by a difference in growth potential of the components of the chimera.

The dermal layer of one cell wide, and the spindle-shaped cell arrangement is only found in the more peripheral parts of the site of fusion, and at these places osmiophilic bodies are frequent to abundant as well (*fig. 2* and *3*). However, their decrease in frequency towards the interior of the ovary and their absence from the dermal cells lining the ovarial cavity (*fig. 2*) is evident. This difference is probably caused by the fact that the dermal cells in the ovarial cavity are of a more distinct meristematic character than the outer cells and, as can also be seen in sections of earlier stages of fusion (*fig. 1*) with distinctly meristematic dermal cells, not showing osmiophilic bodies. The cells on the outside of the ovary are much further in the process of maturation and in these, changing metabolic activities have led to the deposition of phenolic or tannin-like substances modelled as osmiophilic bodies. The enclosed dermal cells form a gradient between the extremes of the inner and outer wall.

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