

Sporoderm morphogenesis in *Euphorbia obesa* and *Croton gratissimus*

M. L. FREAN*

ABSTRACT

Pollen grains of the Euphorbiaceae show a number of pollen types which can be clearly distinguished. Generally different genera are characterized by a specific pollen type. *Euphorbia obesa* Hook. f. and *Croton gratissimus* Burch. subsp. *subgratissimus* (Prain) Burt Davy, represent two genera within the Crotonoideae with different morphology, each type characteristic for the respective genus.

Taxonomically, the genus *Euphorbia* with apetalous flowers consisting of a naked pistil surrounded by several staminate flowers within a cyathium, is considered more advanced than the genus *Croton*. In *Croton* the inflorescence is a raceme with unisexual flowers. The floral whorls of the male show numerous anthers and both calyx and a showy corolla are present. Both genera are insect pollinated.

In both *Euphorbia obesa* and *Croton gratissimus* the pollen wall in section shows columellae, a structure characteristic of angiosperms. However the present ontogenetic studies show that the formation of the columellae differs entirely in the two pollen types. The final stratification of the wall as well as the morphology of the grains differ and evaluation of the exine structure indicates that phylogenetically *Croton* pollen shows more advanced characters than *Euphorbia* — contradicting the floral phylogeny.

This study conducted at light and electron microscope level compares the two pollen types morphologically and ontogenetically, concentrating mainly on the formation of the exine which is tectate-perforate in the prolate tricolpate grain of *Euphorbia obesa* and semi-ectate in the anaperturate, spheroidal grain of *Croton gratissimus*.

The aim of the study was to evaluate the significance of pollen characters in taxonomic and phylogenetic relationships within the Euphorbiaceae. The differing pollen morphology which is related to the taxonomic grouping of tribes within the subfamily (Crotonoideae) emphasizes diversity, which may result from physiological adaptation. The study shows that the same functional end may well be achieved in different ways and this may be a factor underlying the diversity in the heterogeneous family Euphorbiaceae.

RÉSUMÉ

LA MORPHOLOGIE DU SPORODERME CHEZ EUPHORBIA OBESA ET CROTON GRATISSIMUS

Les grains de pollen des Euphorbiacées montrent un nombre de types polliniques qui peuvent être clairement distingués. Généralement, les divers genres sont caractérisés par un type de pollen particulier. *Euphorbia obesa* Hook. f. et *Croton gratissimus* Burch. subsp. *subgratissimus* (Prain) Burt Davy représentent deux genres de Crotonoïdées avec une morphologie différente, chaque type étant caractéristique pour le genre respectif. Taxonomiquement, le genre *Euphorbia* avec ses fleurs apétales constituées d'un pistil nu entouré de plusieurs fleurs staminées dans un cyathium, est considéré comme plus évolué que le genre *Croton*. Chez *Croton*, l'inflorescence est un racème avec des fleurs unisexuées. Les verticilles de fleurs mâles montrent de nombreuses anthères et tant un calice qu'une corolle bien visible sont présents. Les deux genres sont pollinisés par les insectes.

Tant chez *Euphorbia obesa* que chez *Croton gratissimus*, la paroi du pollen, présente en section des columelles, structure caractéristique des angiospermes. Cependant, les études ontogéniques actuelles montrent que la formation des columelles diffère entièrement dans les deux types de pollen. La stratification finale de la paroi, tout comme la morphologie des grains, diffèrent et l'évaluation de la structure de l'exine indique que phylogénétiquement le pollen de *Croton* montre des caractères plus évolués que celui d'*Euphorbia*, à l'opposé de la phylogénie florale.

Cette étude réalisée en microscopie optique et électronique compare la morphologie et l'ontogénie des deux types de pollen, en se basant principalement sur la formation de l'exine, qui est tectée-perforée dans le grain elliptique tricolpé d'*Euphorbia obesa* et semi-ecté dans le grain inaperturé et sphéroïdal de *Croton gratissimus*. Le but de cette étude était d'évaluer l'importance des caractères du pollen dans les affinités taxonomiques et phylogénétiques qui existent au sein des Euphorbiacées. La morphologie différente du pollen, qui est en rapport avec le groupement taxonomique des tribus dans la sous-famille des Crotonoïdées souligne la diversité qui peut résulter de l'adaptation physiologique. Cette étude montre la même résultat peut bien être atteint de différences manières et ceci peut être un facteur responsable de la diversité dans la famille hétérogène des Euphorbiacées.

INTRODUCTION

Cronquist (1968) describes the Euphorbiaceae as being highly diverse in anatomy and pollen morphology. *Euphorbia obesa* Hook.f. and *Croton gratissimus* Burch. subsp. *subgratissimus* (Prain) Burt Davy (referred to as *C. gratissimus* in this study), represent two genera of the heterogeneous family Euphorbiaceae which differ markedly from each other in both their floral structure and pollen

morphology while showing morphologically similar pollen within each genus. Both genera are insect pollinated.

Taxonomists generally consider that the trend toward reduction and aggregation culminates in the pseudanthia (or cyathia) of *Euphorbia*, with flowers consisting of a naked pistil surrounded by several staminate flowers (Cronquist, 1968; Stebbins, 1950). In *Croton gratissimus*, the inflorescence consists of a raceme with many male above a few female flowers. The floral whorls of the male consist of numerous stamens bent sharply inwards and both calyx and a showy corolla are present. Taxonomically, the genus

* Department of Botany, University of the Witwatersrand, 1 Jan Smuts Avenue, Johannesburg 2001, South Africa.

Euphorbia is considered more advanced than the genus *Croton*. Stebbins (1950) thinks it most probable that the flowers of the Euphorbiaceae became apetalous and unisexual as an adaptation to wind pollination, but have reverted to insect pollination.

Pollen of the Euphorbiaceae has been extensively studied at the light level by Punt (1963) and phylogenetic trends in pollen morphology are noted by Webster (1974). Both *Euphorbia* and *Croton* are genera within the subfamily Crotonoideae* which includes a large number of pollen types. The genus *Euphorbia* has pollen with hippomane-configuration, so-called because the tribes Euphorbiae and Hippomaneae in the subfamily Crotonoideae, have almost indistinguishable grains (Punt, 1963). *Croton* is the principal genus among a number of genera of the tribe Crotonae within the Crotonoideae, which show typical croton-configuration. Morphological differences between the pollen types lie chiefly in the configuration of the mature outer wall, the exine, and in the form and arrangement of the apertures.

Euphorbia obesa and *Croton gratissimus* pollen grains conform to the general pattern for angiosperms (see Heslop-Harrison, 1971). The terminology used is that of Faegri & Iversen (1964). The exine of *Euphorbia obesa* is classically tectate-perforate (Fig. 2a). *Croton gratissimus* (Frean, 1972) has a complex ektexine which may be described as semi-TECTATE, since it consists of sculptured projections or clavae, partially joined at tectum level (Fig. 2b). Both exine types are columellate. Walker (1976) considers the most likely evolutionary pathway leading to the development of columellae in angiosperm pollen grains is represented by the sequence tectate perforate to semi-TECTATE. *Croton* with its semi-TECTATE exine represents a more advanced evolutionary trend.

Therefore, a contradiction is found in *Croton gratissimus* where its floral structure may be less advanced than that of *Euphorbia obesa*, whereas its pollen wall stratification shows more advanced features. This study was undertaken with the aim of establishing the possible taxonomic value and evolutionary significance of sporoderm morphogenesis in *Euphorbia obesa* and *Croton gratissimus*. In this context it shows that the final stratification of the pollen wall is achieved in very different ways in these two entomophilous pollen types. The possible significance of these differences is discussed.

METHOD

Developing and mature anthers of *Euphorbia obesa* and *Croton gratissimus* were examined by light, transmission and scanning electron microscopy to show stages of development of the pollen wall and morphology of the mature grain.

Euphorbia obesa was grown in the greenhouse at the University of the Witwatersrand, Johannesburg.

Croton gratissimus was collected from the Daspoort range near Pretoria. Voucher specimens of these two species are lodged with the Moss Herbarium (J), Johannesburg. Other *Euphorbia* and *Croton* spp. were examined by light and scanning electron microscopy to provide confirmation of the generic pollen type. Pollen of *Croton dideroides* M. Denis, *Euphorbia esculenta* Marl., *E. ferox* Marl., *E. gariiepina* Boiss., *E. meloformis* Ait. and *E. splendens* Boj. ex Hook. var. was obtained from the hothouse, Botanical Research Institute, Pretoria; pollen of *Croton zambesicus* Muell. and *C. sylvaticus* Hochst. was taken from specimens in the Moss Herbarium.

Euphorbia obesa

Plants are dioecious, producing 5 rows of bilobed anthers in a cyathium. The anthers mature in ascending order in each row, so that a single cyathium shows anthers and pollen at many stages of development.

Croton gratissimus

Plants are monoecious. The inflorescence consists of a raceme with many male flowers above a few female ones. Anthers are arranged in whorls, the youngest being in the inner-most whorl. Anthers of both species were processed routinely for light, transmission and scanning electron microscopy. The methods used have been previously described by Frean (1972).

Light microscopy

Fresh anthers were squashed in Belling's acetocarmine (Gatenby & Beams, 1950).

For sectioning, entire cyathia of *Euphorbia obesa* and buds of *Croton gratissimus*, stripped of bracts, were fixed in formalinacetic-alcohol, dehydrated in ethanol and tertiary-butyl-alcohol (Johansen, 1940), embedded in wax and sectioned at 5–8 μ m. Longitudinal sections mounted serially on slides, provided a wide range of developmental stages. This material was stained with the periodic acid — Schiff (PAS) reaction for polysaccharides (Humason, 1967) to show cellulosic primexine and intine.

Transmission electron microscopy (TEM)

Anthers were fixed at 4°C in 2% glutaraldehyde in 0,1 M cacodylate buffer at pH 7,3 for 1 hour, postfixed in 1% osmium tetroxide in the same buffer for 1 hour, dehydrated in a graded acetone series and embedded in Luft's (1961) epon.

Sections, cut at 60–90 μ m, were picked up on 200 mesh copper grids and stained with a saturated solution of uranyl acetate in 50% alcohol for 10 minutes, followed by 2 minutes in Reynolds's (1963) lead citrate. They were viewed at 80 KeV in a Jeol JEM 1000 conventional TEM.

Scanning electron microscopy

Mature anthers were processed in a Polaron Critical Point Apparatus E 3800, after routine fixation for transmission electron microscopy. Anthers from herbarium specimens were dissected from *Croton* buds prior to fixation. Critical point

* Editor's note: Crotonoideae = Euphorbioideae.

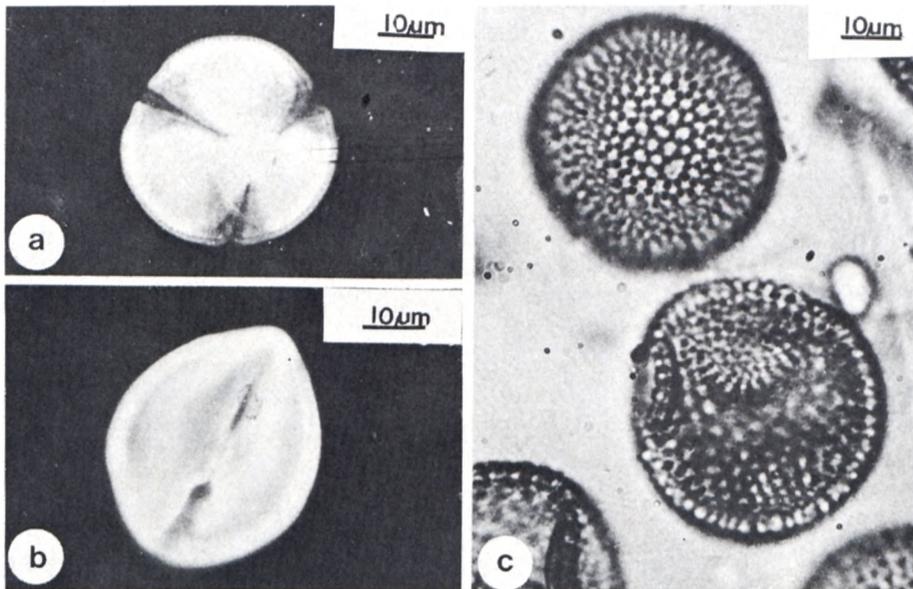


FIG. 1.—Acetolysed grains. a, *Euphorbia obesa*, polar view. b, *E. obesa*, equatorial view. c, *Croton gratissimus*.

dried anthers were broken open onto stubs lightly coated with colloidal graphite, then coated with gold palladium and viewed in an S₄ Cambridge stereo-Scan-EM.

Acetolysis

Resin-embedded sections of material routinely embedded for transmission electron microscopy and mounted on glass slides, were acetolysed on a microscale (Dickinson & Bell, 1972) with a 9:1 acetic anhydride/sulphuric acid mixture. By this technique, combined with the PAS reaction, the stage at which protospollenin became polymerized to sporopollenin was established.

RESULTS

Light microscopy

Acetolysed grains show that at light microscope level, *Euphorbia obesa* conforms to the hippomane configuration, except that it is without a true pore. The grain is tricolpate rather than tricolporate (Fig. 1a, b).

Croton gratissimus shows typical croton-configuration, appearing tectate with clavae rising above the tectum (Fig. 1c), but electron microscopy shows that grains are semi-TECTATE rather than tectate. Grains are spherical and anaperturate.

Transmission electron microscopy

Final wall stratification in grains of *Euphorbia obesa* and *Croton gratissimus* is shown diagrammatically in Fig. 2a, b. The following stages (1–4) are described:

1. Early exine formation

In both species the first sign of activity at the plasma membrane, related to wall formation, occurs at the late pachytene stage of meiosis in the callose enclosed microspore mother cell (Fig. 3a, b). A matrix layer appears outside the plasma membrane, closely associated with distended ends of rough endoplasmic reticulum in the cytoplasm. This layer

is possibly mucopolysaccharide in nature. Within this layer the PAS-positive, cellulosic primexine is laid down, serving as a template within which the ektexine elements form.

2. Growth of the exine elements

In *Euphorbia obesa* further development of the ektexine takes place at the tetrad stage. At the surface of the primexine, flask-shaped vesicles appear (Figs 3c & 4c). They appear to move out with the primexine as it forms. These vesicles are so arranged that by excluding both the formation of the primexine fibrils and the laying down of the ektexine in these regions, they form the perforations of the tectum.

Concomitantly with the formation of the vesicles and the primexine, columellae form on specialized areas of the plasma membrane (Fig. 4a, c). By the end of the tetrad stage the bases of the columellae are joined by the foot-layer (Fig. 4c). The ektexine now is PAS-positive, but not acetolysis resistant, and resembles the early matrix material in texture (?mucopolysaccharide). It is composed of protospollenin with some final polymerisation evident

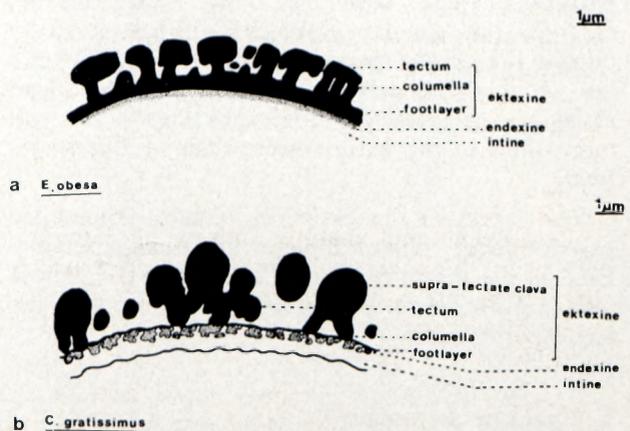


FIG. 2.—Diagrammatic representation of the mature pollen wall as seen in section by transmission electron microscopy. a, *Euphorbia obesa*. b, *Croton gratissimus*.

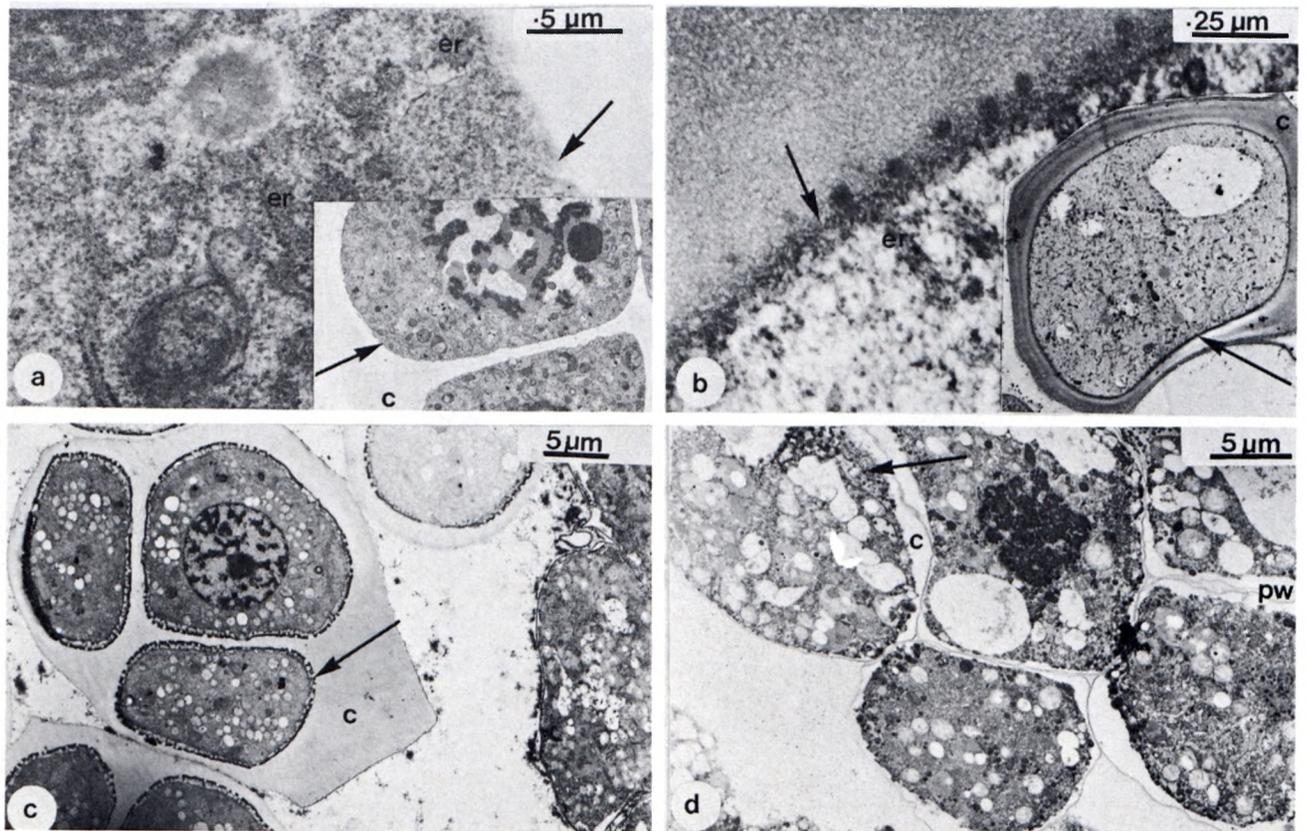


FIG. 3.—Early wall formation. a, *Euphorbia obesa*, matrix material is seen at the plasma membrane (arrowed). Inset: microspore mother cell, pachytene stage, wavy plasma membrane (arrowed) is characteristic of early wall formation; c = callose. b, *Croton gratissimus*, matrix material (arrowed) is seen at plasma membrane with early ectexine elements. Inset: microspore mother-cell, early signs of wall formation are visible (arrowed); c = callose. Note that in both grains elements of the endoplasmic reticulum (er) with distended cisternae are associated with early events outside the plasma membrane. c, *E. obesa*, callose-surrounded tetrad, ectexine elements arrowed; c = callose. d, *C. gratissimus*, late stage of microspore mother cell, ectexine elements arrowed; c = callose, pw = primary wall.

in the osmiophilic (lipoidal) regions of the ectexine (Fig. 4c).

At this time the apertural regions of the microspore are defined. In the central colpus area (there is no true pore) a plug of matrixlike material is formed (Fig. 5c).

In *Croton gratissimus* development of the ectexine takes place largely during the microspore mother cell stage. (Fig. 3d). Globular outgrowths seen in stage 1 (Fig. 3b) enlarge. They appear to consist of lipid surrounded by ribosome-like particles. These structures, the future clavae, enlarge, but smaller globules continue to form outside the plasma membrane (Fig. 4b, d). They are not strongly PAS-positive and show early acetolysis resistance, suggesting a different composition with more lipid in the early stages, than in *Euphorbia obesa*.

The layers of the ectexine, tectum, columellae and footlayer, now complete, are finally polymerised in the thecal fluid on release from the callose wall in both grains. In this they are typical of many angiosperm grains.

3. Endexine formation

In both grains electron lucent lamellae of unit membrane core dimensions, on which osmiophilic material (?sporopollenin) accumulates, separate

endexine and ectexine. In *Euphorbia obesa* the osmiophilic material forms an endexine element outside the plasma membrane. Movement of this element through an apparent space separating ect- and endexine, results in its apposition to the lower surface of the foot-layer. Here a number of elements are applied, become compacted and form a homogeneous layer. Evidence that they are membrane derived, remains in the form of lucent cores (Fig. 4e) or 'white lines' (4–5 nm). During this period the apertural plug is transformed into the lamellate structure of the mature aperture (Fig. 5d) by the invasion of protoplasmic tubules.

In *Croton gratissimus* ribosome-coated globules fill the endexine space (Fig. 4f). These globules polymerise to form electron dense bodies in an inter-bedded zone which consists of endexine and intine (Fig. 5e). The derivation of the electron lucent lamellae or 'white lines' was not traced in this grain.

4. Intine formation

Presence of the intine layer is confirmed by the PAS-reaction confirming its cellulosic nature. A special feature of the intine in *Euphorbia obesa* lies in the formation of intine reservoirs on each side of the central aperture (see inset, Fig. 5a). In *Croton gratissimus* two layers can be distinguished in the intine — an inner layer with cytoplasmic inclusions in the fibrillar ground substance and an outer layer forming the inter-bedded zone. Vesicles, apparently

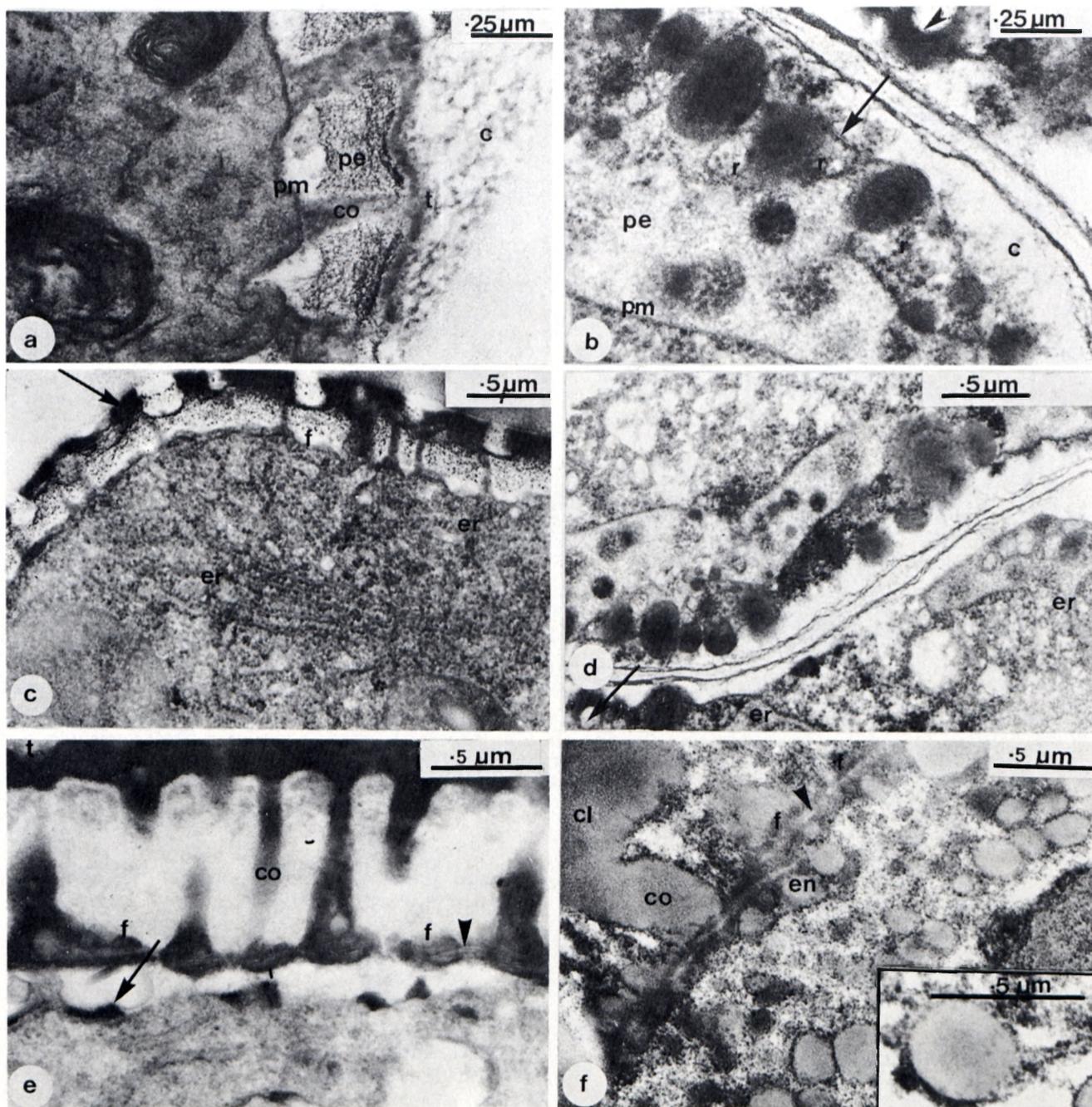


FIG. 4.— Growth of ektexine elements. a, *Euphorbia obesa*, ektexine elements viz columellae (co) and tectum (t) showing finely textured appearance; c = callose, pe = primexine, pm = plasma membrane. b, *Croton gratissimus*, globular outgrowths (clavae) some with lucent cores (arrowhead) appear to be ribosome surrounded (r) and show intimate association with membranes of unit membrane dimensions (arrowed), cf with plasma membrane (pm) at the cytoplasm surface; c = callose, pe = primexine, pm = plasma membrane. c, *E. obesa*, microspore cytoplasm with ektexine. Note arrays of tubular, rough endoplasmic reticulum (er), polymerizing osmiophilic regions of the tectum (arrowed), and formation of the footlayer (f). d, *C. gratissimus*, microspore cytoplasm with ektexine. Note tubular ribosome coated endoplasmic reticulum (er) associated with the plasma membrane, and electron lucent cores of clavae (arrowed, lower left corner). e, ektexine polymerization and endexine formation. *E. obesa*, ektexine showing evidence of polymerization in enhanced osmiophilia. Note that some areas remain unpolymerized (less electron dense), at the plasma membrane an endexine element (arrowed) is forming, while a second element above it is being applied to the footlayer (f) of the ektexine, white lines are visible in the endexine (arrowhead); co = columella, t = tectum. f, *C. gratissimus*, growth and polymerization. Note columella (co), footlayer (f) and globular elements of the endexine (en). Inset: small ektexine globule.

empty, appear in the inter-bedded zone and among the ektexine elements as final wall stratification is achieved. These resemble 'fusiform vesicles' as described by Rowley & Erdtman (1967) in *Populus* and represent the remains of cytoplasmic membranes.

Scanning electron microscopy

Fig. 6 shows the surface morphology of mature grains of *Euphorbia obesa* with a tectum perforatum and *Croton gratissimus* with clavae at supra-TECTAL level.

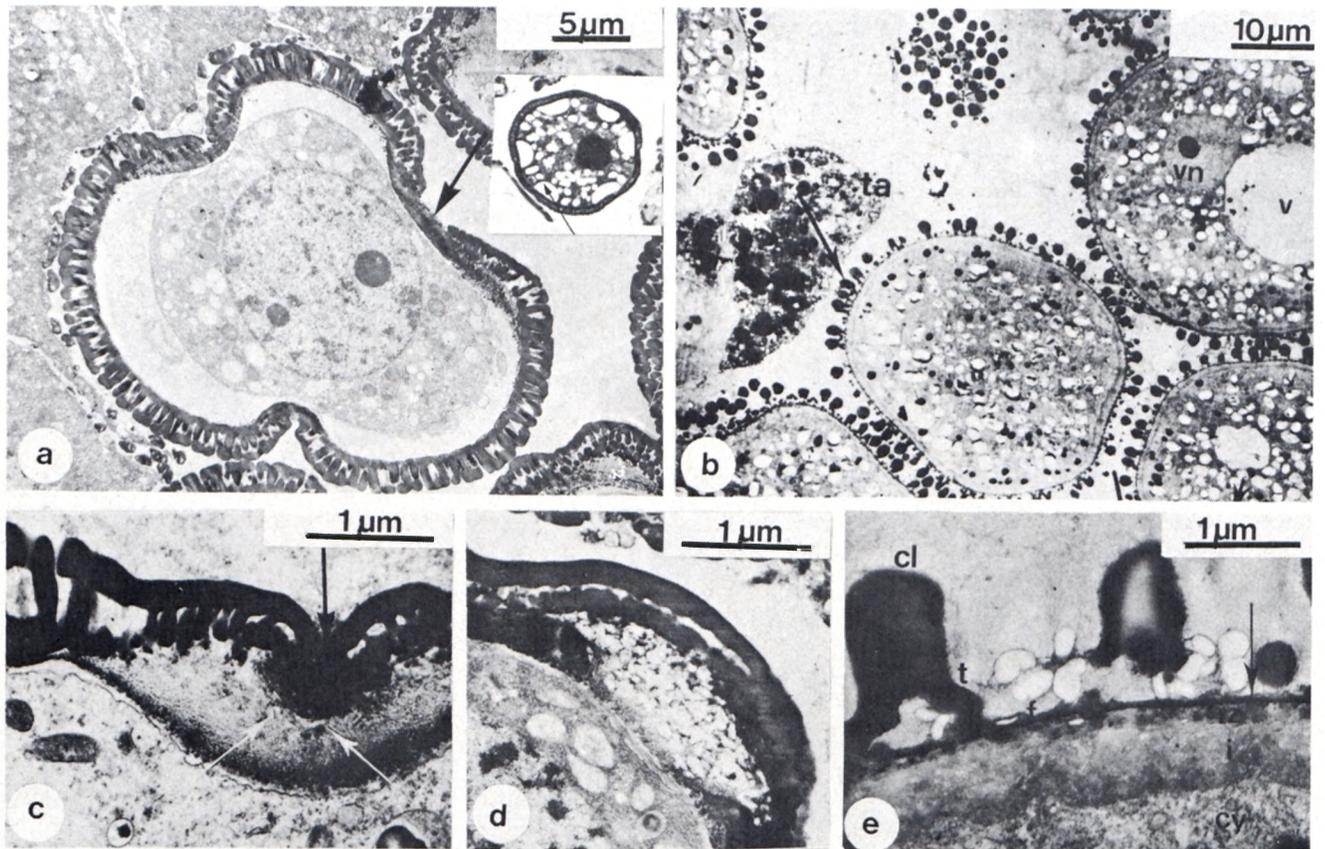


FIG. 5.—Final exine stratification and intine formation. a, *Euphorbia obesa*, apertures arrowed, inset shows mature grain with intine reservoirs (arrowed). b, *Croton gratissimus*, tectate areas arrowed, clavae rise above tectum. c, *E. obesa*, fibrillar material of aperture shows apertural exine (black arrow) and invasion of fibrillar plug by protoplasmic tubulus (white arrows). d, *E. obesa*, final lamellate appearance of aperture. e, *C. gratissimus*, final appearance of the pollen wall; cl = clavae, f = footlayer, t = tectum, i = intine, iz = interbedded zone, fusiform vesicles arrowed, cy = cytoplasm.

DISCUSSION

This investigation shows that *Euphorbia obesa* and *Croton gratissimus* differ morphologically in the stratification of the sporoderm and morphogenetically in its formation. Both grains are columellate, a feature considered to be characteristic of angiosperms (Walker, 1976). However, achievement of columella formation differs markedly in these two taxonomically related genera.

1. Ontogeny

(a) Formation of the columellae (ektexine)

In *Euphorbia obesa* the plasma membrane appears to influence exine form in providing recognition sites at which the columellae form (Fig. 4a). Such definitely defined sites were not seen in *Croton gratissimus*, and plasma membrane involvement is not as clearly delimited.

In contrast, involvement of membranes in ektexine formation is dramatically evident outside the plasma membrane during ektexine formation in *C. gratissimus*. Although their actual derivation from the plasma membrane or the cytoplasm is obscure, their dimensions (10 µm in width) conform to those of the plasma membrane (Fig. 4b). These membranes are associated with globular elements of the ektexine, and with ribosomelike particles. The unusual occurrence of ribosomelike particles on the exine-forming side of the plasma membrane is

outside the scope of this discussion. The globular elements increase in size during this stage.

Lamellae of unit membrane dimensions have been described in exine formation in other grains, e.g. in *Lilium* (Dickinson & Heslop-Harrison, 1968). However, the situation in *Croton gratissimus* where a system of growth by apposition of material from the inside is suggested (see also *Polyceratocarpus* in the Annonaceae, Le Thomas & Lugardon, 1976) is very different from the precisely controlled development of a typical columellar exine as seen in *Euphorbia obesa* or *Lilium*, which in turn differ from each other in the precise mechanism of columella formation.

The characteristic pattern of the pollen wall in flowering plants is established by the maternal cytoplasm of the pollen mother cell (Rogers & Harris, 1969; Heslop-Harrison, 1971). The mechanism by which the cytoplasm forms the pattern is not clear. However, it does seem clear, even from the limited number of pollen types dealt with in this discussion, that although columellae are characteristic of angiosperm pollen and are considered to serve a common purpose in providing structure which increases exine flexibility and houses sporophytic proteins (Muller, 1979; Heslop-Harrison, 1971), they differ in construction and ontogeny.

(b) Endexine formation

Although the final appearance of the endexine differs greatly in *Euphorbia obesa* and *Croton*

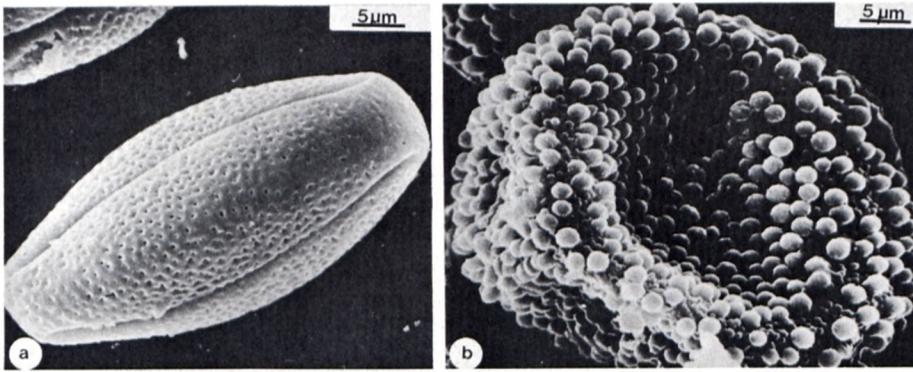


FIG. 6.—Scanning electron micrographs. a, *Euphorbia obesa*, b, *Croton gratissimus*.

gratissimus (Fig. 5e, f), membrane involvement remains evident in the form of 'white lines' of unit membrane core dimensions. Formation of the endexine appears to follow an established angiosperm pattern as described in many grains e.g. *Lilium* (Dickinson, 1976). The term 'white lines' is used by Godwin, Echlin & Chapman (1967) in their description of endexine formation in *Ipomoea*.

In *Croton gratissimus* the interbedded endexine appears to afford the grain a capacity for expansion provided by the lamellate apertures in *Euphorbia obesa*.

The ubiquitous mode of formation of the endexine (when present) in angiosperms is consistent with the rôle of a layer perhaps more limited in function than the ectexine where adaptation to environmental influences such as dehydration, compatibility, pollen vectors, may influence more strongly the evolution of characteristic exine patterns.

(c) Intine formation

In *Euphorbia obesa* the intine reservoirs may provide extra sites to house enzymes (Knox & Heslop-Harrison, 1970) since this layer in other parts of the grain is extremely thin. They also have a harmomegathic function in accommodating grain expansion.

In *Croton gratissimus* the thick intine may provide additional enzyme sites, compensating for the rather open, semi-TECTATE ectexine.

In concluding this section of the discussion it can be stated that in many ways these two pollen types appear to be achieving the same end point despite wide differences in pollen ontogeny and morphology.

2. Morpho-physiological adaptation

A further comparison which can be made between these two grains is their response to hydration. Both show a great capacity for expansion. In *Euphorbia obesa* the accommodation mechanism for expansion of this tricolpate grain lies in the granular endexinous apertural floor, apertural lamellations and in the position occupied by two intinous reservoirs, one on each side of the presumptive pore; there is no true pore. *Croton gratissimus* in common with many Crotonaeae, has large spherical pollen grains ($\pm 50 \mu\text{m}$ in diameter), is aporate and

has a semi-TECTATE exine with massive supra-TECTATE projections. Breckon & Webster (1974) show that when the grain is turgid the tectal pegs spread apart exposing the layers below while dehydration results in shrinkage pulling the tectal pegs together, with their apices forming a continuous ECTEXINE which may contribute to the elasticity of the exine in these aporate *Croton*-type grains. The thin inter-bedded endexine may contribute to this elasticity.

Whatever the cause of these adaptive modifications of the sporoderm morphology to changes in physiological conditions, the resulting features must underlie some of the taxonomic heterogeneity of this family. In relating morphological wall features to current taxonomic and phylogenetic concepts, it is noted that the number of species examined is small. However, the members of the genus *Euphorbia* and of the genus *Croton* which are compared, are

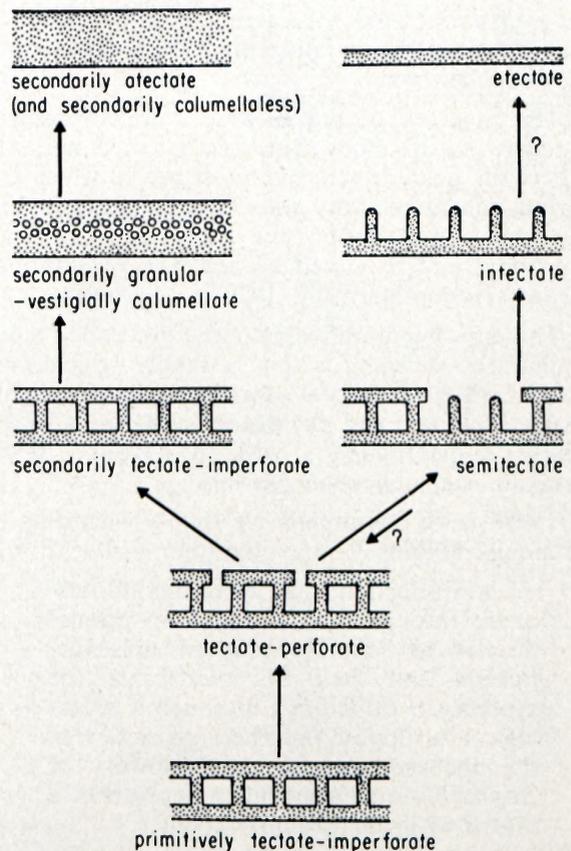


FIG. 7.—Diagram of evolutionary trends within the angiosperms (Walker, 1976).

representative of their morphological types or 'configurations' as described by Punt (1963).

3. Evolutionary status of the pollen wall

The exine of *Euphorbia obesa* is classically tectate-perforate (Figs 2a, 5a & 6a). *Croton gratissimus* has a complex ectexine which may be described as semi-TECTATE, since it consists of sculptured projections or clavae partially joined at tectum level (Figs 2b, 5b & 6b). Both exine types are columellate. Walker (1976) considers that the most likely evolutionary pathway leading to the development of columellae in angiosperm pollen grains is represented (as shown in Fig. 7) by the sequence tectate-perforate to semi-TECTATE. *Croton* with its semi-TECTATE exine would thus represent a more advanced evolutionary trend.

Walker suggests that although there is no strong correlative evidence to suggest that pollen which has already reached the semi-TECTATE stage can reverse back to tectate-perforate, future studies may indicate that such a reversal can occur. Within the boundaries of present concepts this study shows that a contradiction is found in *Croton gratissimus* where its floral structure may be less advanced than that of *E. obesa*, whereas its pollen wall stratification may show more advanced features.

FINAL REMARKS

This study achieves its main stated aim, namely that of examining and describing ontogeny and morphological stratification of the sporoderm in *Euphorbia obesa*, and *Croton gratissimus* subsp. *subgratissimus*, and indicates the importance of ontogenetic studies in interpreting the final structure of the pollen wall.

The diversity of pollen walls reflects adaptive function. As this study shows, pollen walls not only reflect the possible selective pressures to which the various functions of the grain have been exposed in the course of evolution, but shows that the same functional end may well be achieved by different means (Heslop-Harrison, 1979).

The differing morphology of the two pollen types emphasizes diversity within a family which may result from physiological adaptation, and highlights some taxonomic and phylogenetic problems in the family Euphorbiaceae, with its diverse floral structure and pollen morphology.

There is no comprehensive theory according to Cronquist (1968):

'of how selection could drive the inflorescence through a full cycle from normal flowers to separate reduced unisexual flowers and then to pseudanthia (or cyathia) in *Euphorbia*, although it seems logical to suppose that the organisation of the inconspicuous reduced flowers of *Euphorbia* into a cyathium represents a return to ordinary entomophily'.

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