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Embryo sac development in some South African *Lantana* species (Verbenaceae)

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ABSTRACT

Evidence that the South African *Lantana camara* L. complex only produces sexual embryo sacs is provided. It is shown that the archesporium occasionally divides mitotically and that both archesporia form tetrads. The chalazal megaspore of one tetrad and the micropylar megaspore of the second tetrad develop into *Polygonum* type embryo sacs.

L. rugosa Thunb. also forms *Polygonum* type embryo sacs. The *L. rugosa* embryo sac has a much more densely packed cytoplasm, smaller vacuole and the position of the polar nuclei differs from that of the *L. camara* embryo sac. It is possible to distinguish between *L. camara* and *L. rugosa* on their embryo sac morphology alone.

INTRODUCTION

The genus *Latana* is represented in South Africa by *L. camara* L., *L. mearnsii* Moldenke, *L. montevidensis* (Spreng.) Briq., *L. rugosa* Thunb. and *L. trifolia* L. *L. camara* is an introduced plant that has escaped from cultivation and has become an aggressive invader of the warmer subtropical regions of South Africa. Of the indigenous *Lantana* species, *L. rugosa* is the most abundant and widespread.

The occurrence of apomixis in *L. camara* has been inferred from morphological studies of F_1 plants (Raghavan & Arora, 1960; Khoshoo & Mahal, 1967), although no evidence of apomixis was obtained by embryological studies (Junell, 1934; Patermann, 1935; Tatachar, 1940; Padmanabhan, 1959; Khaleel & Nalini, 1972).

Recently, Spies & Stirton (1982c) have described the occurrence of two embryo sacs per ovule in *L. camara*. One of these embryo sacs was found to be sexual (resulting from a meiotic division of the archesporium), whereas the origin of the other embryo sac was, at that time, not determined. The aim of this study is to examine the mode of origin of the second embryo sac and to compare embryo sac development in the exotic *L. camara* and the indigenous *L. rugosa*.

MATERIALS AND METHODS

The plants used during this study were naturalized plants collected in the field throughout South Africa and transplanted in the Pretoria National Botanical Garden. The study sample includes all material used in the previous study (Spies & Stirton, 1982c) and, in addition, *Spies* 750, 751, 752, 765, 767, 797, 834, 836, 851, 854, 857 and 887, and *Stirton* 8928 and 9834 were examined. *L. rugosa* was represented by *Spies* 1056 and 1060. Herbarium voucher specimens are kept in the National Herbarium, Pretoria (PRE).

Young inflorescences were fixed in Navashin fixative (Stockholm modification — Maheshwari, 1939) at 4°C for at least 24 hours. The material was dehydrated in an ethyl alcohol and tertiary butyl alcohol series and subsequently embedded in Tissue Prep (T565). Serial sections (7–10 μ) were stained in Safranin (Johansen, 1940) and Fast Green (Sass, 1951).

RESULTS AND DISCUSSION

(a) Lantana camara

Lantana camara has a bilocular ovary with a single anatropous ovule per locule. A single massive integument surrounds the uniseriate nucellus. The archesporium is hypodermal and either functions directly as a megaspore mother cell (Fig. 1a) or divides mitotically (Fig. 1i) and subsequently acts as two megaspore mother cells. The further development of the single archesporium (Figs 1a – g, & 2a, c, d) has been described elsewhere (Spies & Stirton, 1982c).

In those instances where two archesporia are present, both cells divide meiotically to form two parallel linear tetrads of megaspores (Figs 1j & 2b). The chalazal megaspore is always the functional megaspore when only one tetrad is present (Spies & Stirton, 1982c), but the chalazal megaspore of one tetrad and the micropylar megaspore of the second tetrad are functional when two tetrads are present (Figs 1k & 2b). As further development of the megaspores into embryo sacs progresses, a difference in developmental stage between the two embryo sacs is distinguishable. The embryo sac originating from the chalazal megaspore is always developmentally ahead of the embryo sac that originated from the micropylar megaspore (Fig. 1l – n). At maturity both embryo sacs eventually resemble the sexual *Polygonum* type embryo sacs (Maheshwari, 1950) with identical polarization (Fig. 2f, g).

During the early stages of embryo sac development no differentiation of nuclei is visible (Fig. 2c,

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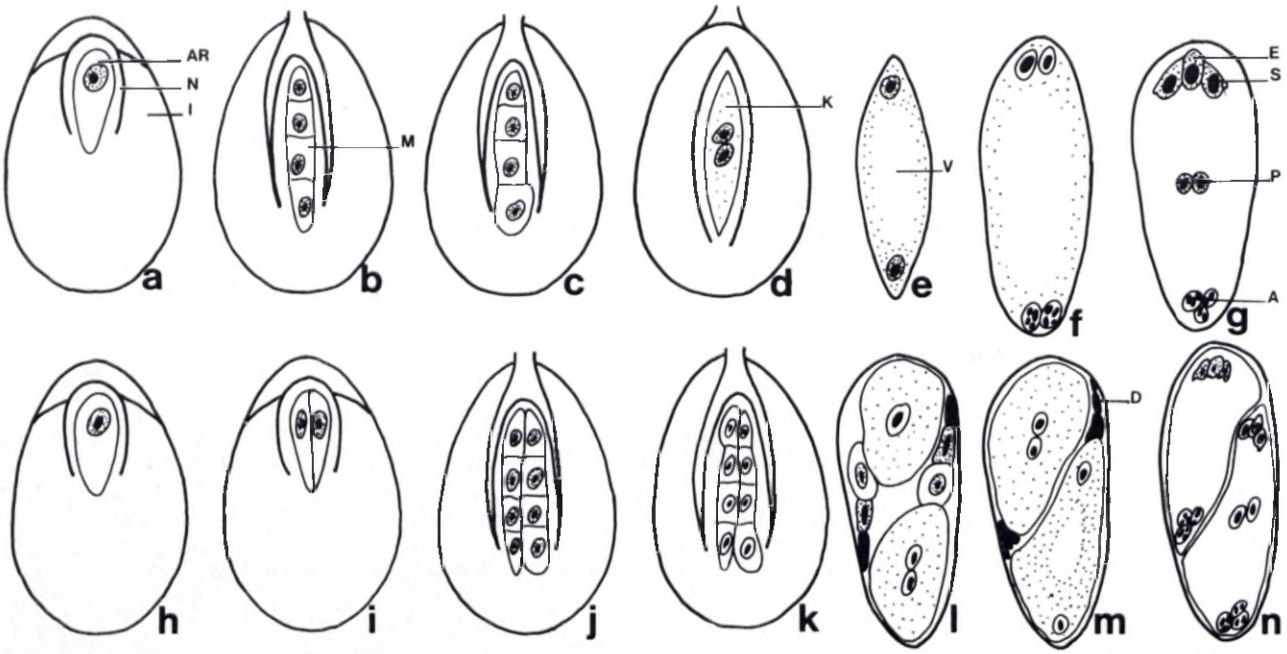


FIG. 1.—Schematic representation of embryo sac development in *Lantana camara*. a, archesporium; b, tetrad; c, tetrad with chalazal megaspore development, d, 2-nucleate embryo sac; e, 2-nucleate embryo sac where the formation of a vacuole pushed the nuclei to the opposite poles; f, 4-nucleate embryo sac; g, mature *Polygonum* type embryo sac; h–n, same as a–g, except mitotic division of the archesporium occurred first and two embryo sacs are formed. Abbreviations: A, antipodal cell; AR, archesporium; D, degenerating cell; E, egg cell; I, integument; K, embryo sac; N, nucellus; M, megaspore; P, polar nuclei; S, synergid; V, vacuole.

d). All nuclei are round to oval with one large nucleolus. The chromatin stains slightly darker towards the membrane. The nuclear morphology remains unchanged until after the 4-nucleate stage. Differentiation of the nuclei occurs during the 8-nucleate stage.

The antipodal nuclei resemble the undifferentiated nuclei, except for the number of nucleoli sometimes formed. These nuclei often have more than one small nucleolus. Whereas the antipodal nuclei may be slightly elongated or elliptical, the polar nuclei are usually round (Fig. 2f, g). Since the cytoplasm is not as dense in the central region of the embryo sac as in the polar regions, the polar nuclei appear clearer and better defined than the micropylar or antipodal nuclei. Several nucleoli of different sizes can occasionally be seen in each polar nucleus.

Various numbers of nucleoli might be a result of different polyploid levels. Various polyploid levels for *Lantana camara* in South Africa have been described (Spies & Stirton, 1982a & b; Spies, 1984) and as a result of polyploidy there may be a greater number of nucleoli (Garber, 1950). Therefore, the correlation between the number of nucleoli per nucleus and the polyploid level of the plant might provide an interesting study.

An egg cell surrounded by two synergids formed the egg apparatus (Fig. 2g). The egg cell is oval to rounded with a single large nucleolus and a vacuole on the micropylar side of the nucleolus. The synergids are more elongated and surround the egg cell on three sides and each synergid has a prominent hook on the side facing away from the egg cell. Usually only one large nucleolus per synergid can be seen. This nucleolus is separated from the chalazal membrance of the synergid by a vacuole. The cytoplasm of the synergids stains somewhat darker than the cytoplasm of the egg cell.

The cytoplasm of the embryo sac is denser at the poles than in the centre, with the micropylar pole more dense than the chalazal one. The greater part of the embryo sac consists of a very large vacuole. The polar nuclei are usually suspended in cytoplasm in the central part of the embryo sac next to the cell membrane (Fig. 2e–g).

(b) Lantana rugosa

Lantana rugosa also has a bilocular ovary with a single anatropous ovule per locule. A single massive integument surrounds the uniseriate nucellus. The archesporium is hypodermal and functions directly as a megaspore mother cell (Figs 3a & 4a). Embryo sac development (Fig. 3a – g) corresponds with that of *L. camara* except that no case of the development of more than one tetrad or embryo sac was seen in either plant studied.

Although the development of the embryo sac is similar in *L. camara* and *L. rugosa*, the *L. rugosa* embryo sac can be distinguished morphologically from that of *L. camara* at all stages. The main distinction lies in the density and staining of the cytoplasm. *L. rugosa* has a much denser and darkly stained cytoplasm than *L. camara* at all developmental stages of the embryo sac (compare Figs 2 & 4).

Undifferentiated nuclei in *L. rugosa* are round with a single, large nucleolus. The density of the chromatin increases towards the nuclear membrane (Fig. 4b, c). This nuclear morphology persists till after the 4-nucleate stage.

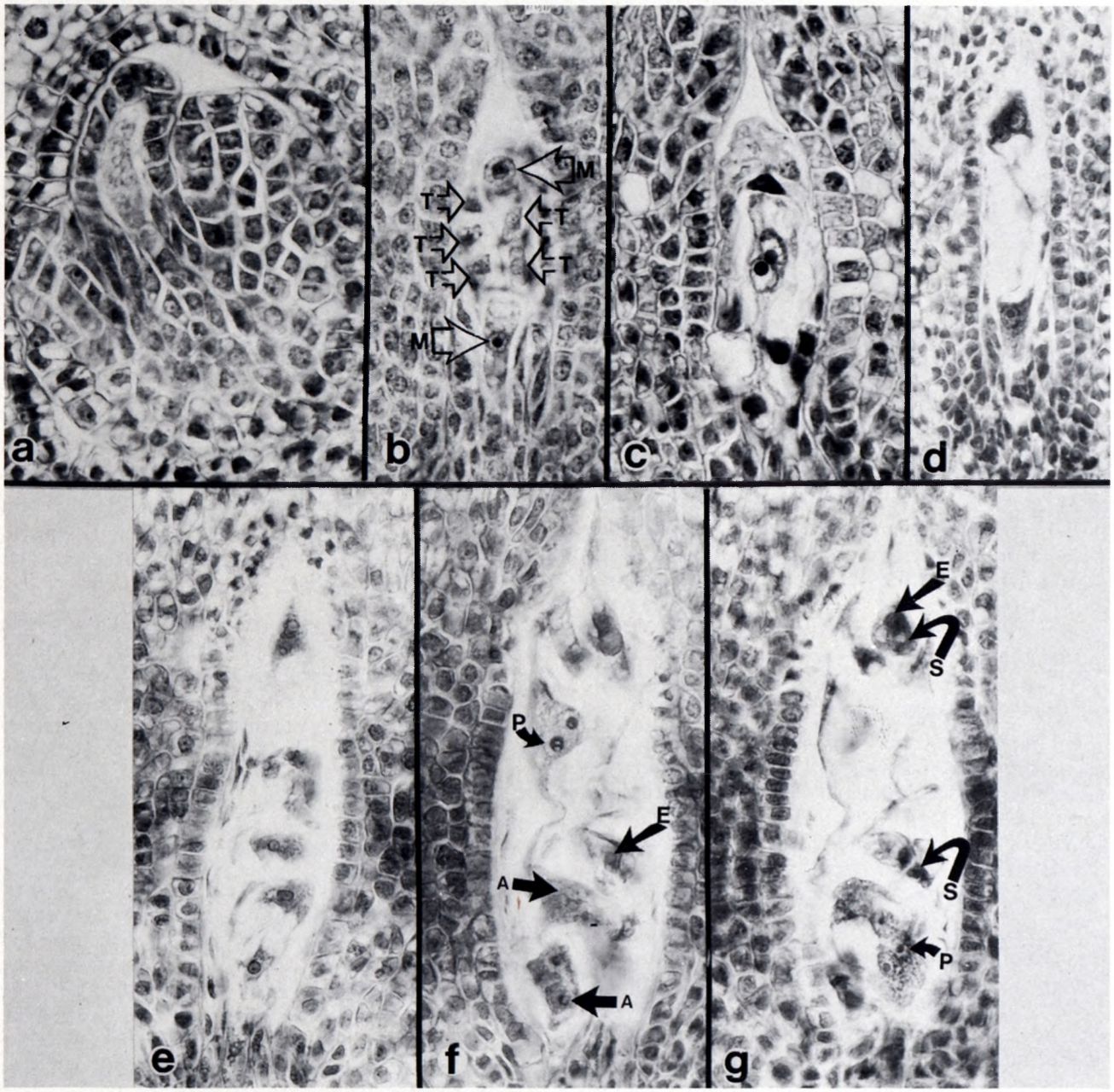


FIG. 2.—Photomicrographs of embryo sac development in *Lantana camara*. a. archesporium; b. twin tetrads, initialization of the development of two embryo sacs (M, developing megaspore; T, nonfunctional megaspores); c, 2-nucleate embryo sac; d, 4-nucleate stage; e, 2 young 8-nucleate embryo sacs; f & g, 2 mature embryo sacs per ovule (breakage of embryo sac membrane during preparation makes interpretation difficult). × 420. See Fig. 1 for legend to abbreviations.

During the 8-nucleate stage the antipodal cells start to degenerate and integrate with the integument to such an extent that these cells cannot be recognized as antipodal cells (Fig. 4e–g). This also results in the embryo sac being fused to the integument. When this integration process starts, each antipodal cell has a large nucleus with a single small nucleolus and scattered chromatin regions throughout the nucleus. The antipodal cells vary from round to elliptic, elongated and conical.

The polar nuclei are round with a single large nucleolus in each (Fig. 4f, g). Although the polar nuclei resemble the undifferentiated nuclei, the nucleolus of the polar nucleus is darker stained than in the undifferentiated nuclei. The polar nuclei are situated in the proximity of the egg apparatus and not in the central part of the embryo sac as in *L. camara*.

The egg apparatus contains the egg cell and two synergids. The egg cell is broad pear-shaped and a single large nucleolus can be seen (Fig. 4f). In cross-section the synergids have a more or less round body with a long beak-shaped protrusion (Fig. 4f). The nucleus is situated on the micropylar side of a vacuole in the chalazal side of the synergids. Large numbers of vacuoles dispersed throughout the synergid are occasionally found (Fig. 4f).

The cytoplasm is stained much darker and is denser than in *L. camara*. The large vacuole between the polar nuclei and the antipodal cells is surrounded by a dense cytoplasmic layer adjacent to the cell membrane. Occasionally one or two small

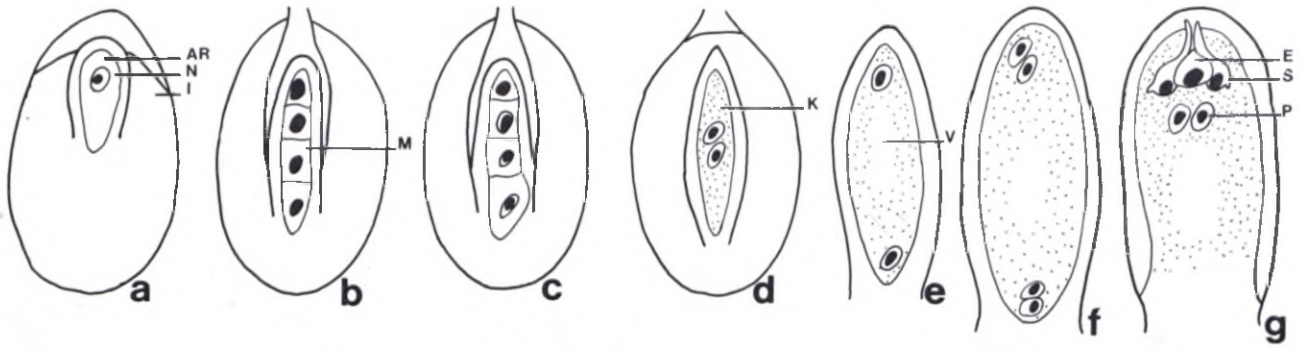


FIG. 3.—Schematic representation of embryo sac development in *Lantana rugosa*. a. archesporium; b, tetrad; c, tetrad with chalazal megaspore development; d, young 2-nucleate embryo sac; e, old 2-nucleate embryo sac; f, 4-nucleate embryo sac; g, mature embryo sac. See Fig. 1 for legend to abbreviations.

vacuoles occur between the polar nuclei and the egg apparatus (Fig. 4d). When these vacuoles are present, they are surrounded by a very thick cytoplasmic layer. In some embryo sacs the polar nuclei and egg apparatus are adjacent with no vacuole present between them. As a result of the denser cytoplasm the whole embryo sac appeared more solid and stable than that of the flimsy *L. camara* embryo sac which usually broke somewhere during the cutting or staining process.

(c) *Comparison with other Verbenaceae*

All members of the Verbenaceae, that have been examined embryologically, have a *Polygonum* type of embryo sac development (for a list of contributors — see Davis, 1966). A single exception is *Avicennia officinalis*, which has an *Allium* type embryo sac development (Karsten, 1891). Karsten's report has not been confirmed to date.

The occurrence of more than one archesporium per ovule has been described in *Pityrodia bartlingii* (Junell, 1934), *Premna integrifolia* (Patermann, 1935), *Lippia nodiflora* (Pal, 1951), *Lantana camara* (reported as *L. aculeata* — Khaleel & Nalini, 1972) and is now confirmed for *L. camara*. Khaleel & Nalini (1972) distinguished up to three archesporia in *L. camara*. Although only one usually developed while the rest degenerated, two tetrads were occasionally seen in their material. The present study indicated a much higher frequency (up to 100% in some plants) of twin tetrads in the South African *L. camara* complex.

Frequently more than one megaspore per tetrad developed in *Lantana camara*, *L. involucrata*, *Bouchea incrassata*, *Petrea volubilis* (Junell, 1934), *Citharexylum ilicifolium* (Patermann, 1935) and seldom in *Lantana indica* (Tatachar, 1940). In addition to this phenomenon, Patermann (1935) observed that, although only one megaspore developed in *Avicennia officinalis*, the rest did not degenerate. The same phenomenon was observed during this study in *Lantana camara*.

It will be interesting to examine earlier stages of embryonic development in plants that developed more than one megaspore. Such a study would indicate whether all developing megaspores originated from the same tetrad or whether different archesporia formed different tetrads. In addition, such a study would show whether each tetrad has only one functional megaspore.

Very little information exists in the literature on the morphology of Verbenaceae embryo sacs. The embryo sac appears to be usually elongated elliptical with the micropylar region broader than the chalazal end as seen during this study.

Schnarf (1931) studied embryo sac development in the Angiospermae and concluded that the three small antipodal cells degenerate at an early stage. Junell (1934) demonstrated that the antipodal cells in the Verbenaceae were different and often divided to form many more cells. This phenomenon was also observed in *Clerodendron phlomidis* (Misra, 1937) and *Lantana indica* (Tatachar, 1940). Contrary to this, both Patermann (1935) and Pal (1951) confirmed Schnarf's (1931) observation in other representatives of the Verbenaceae.

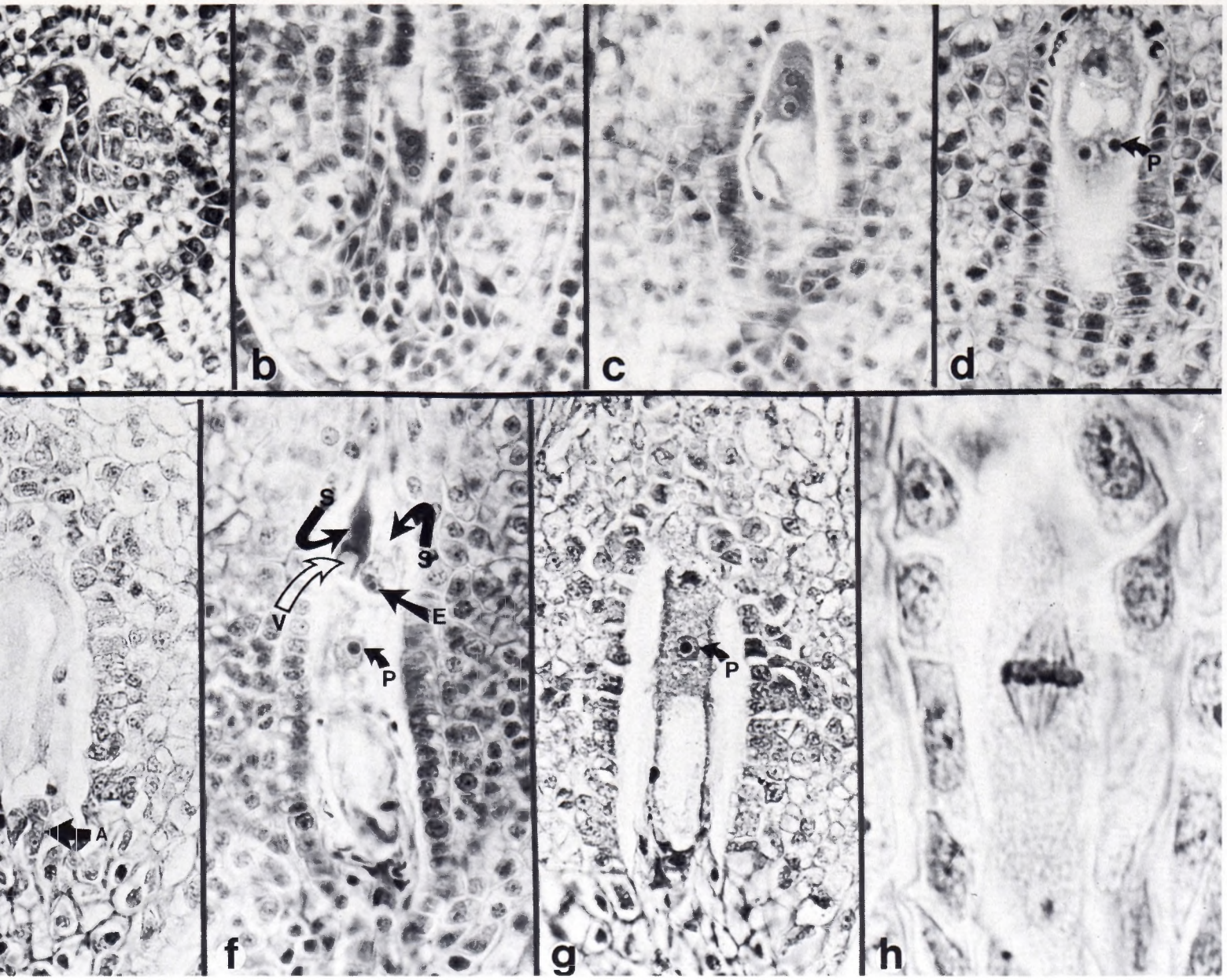
During the present study *L. camara* was observed to have three small antipodal cells which persisted until at least the completion of the formation of the egg apparatus. The early degeneration of the antipodals was observed in *L. rugosa*. The degeneration or persistence of antipodal cells is, therefore, not a constant characteristic of the Verbenaceae.

The egg cell in the Verbenaceae is oval to flask-shaped and a large vacuole in the micropylar region was first observed by Misra (1939). In contrast the pear-shaped synergids have a large vacuole in the chalazal part (Misra, 1939). The synergids are prominently hooked and a beak-shaped apex was described in *Clerodendron phlomidis*, *Caryopteris wallichiana* (Misra, 1939), *Lippia nodiflora* (Pal, 1951) and *Lantana rugosa* (present study). The present study confirmed hooked synergids in *Lantana camara*, but the beak-shaped apex was not as prominent as in *L. rugosa*.

This study has, therefore, indicated that, although similarities in embryo sac development exist in the Verbenaceae, differences can even be found at specific level.

CONCLUSIONS

The reproduction studies of Raghavan & Arora (1960) and Khoshoo & Mahal (1967) indicated



otomicrographs of embryo sac development in *Lantana rugosa*. a, archesporium; b & c, 4-nucleate embryo sac; d – g, mature embryo sac; h, metaphase plate of aclear division in embryo sac. (a – g = × 470 and h = × 1170.) See Fig. 1 for legend to abbreviations.

matroclinous progeny in open pollinated *L. camara*. Their assumption that the matroclinous progeny represented at least facultative apomixis is not supported by any cytological study. The only study that might have supported an apomictic development was the one by Spies & Stirton (1982c), where a second embryo sac of unknown origin was described. The current study proves that the second embryo sac also has a sexual origin. Therefore, no cytological evidence for apomixis in any *Lantana* species has yet been presented.

Monosporic 8-nucleate embryo sacs, known as the *Polygonum* type embryo sac, occur in at least 70% of studied Angiospermae (Maheshwari, 1950). The formation process is similar in all these plants but the final product may differ significantly. Davis (1966) discussed the differences in embryo sac development and embryo sac components as a means to help in the taxonomic treatment of taxa. However, she did not indicate at what taxonomic level this criterion could be used.

The morphological differences between the embryo sac components of *L. camara* and *L. rugosa* indicate that embryo sac studies may contribute to taxonomic separation at the specific level. However, these morphological differences may be an indication of greater phylogenetic differences than presently assumed and more studies of closely related species are necessary to test the taxonomic effectiveness of this approach.

UITTREKSEL

Bewyse word gelewer dat die Suid-Afrikaanse Lantana camara *L. kompleks slegs geslagtelike kiemsakke vorm. Daar word aangetoon dat die archespoor soms mitoties deel en dat beide archespore tetrades vorm. Die chalazale megaspoor van die een tetrade en die mikropilêre megaspoor van die ander tetrade ontwikkel in* Polygonum *tipe kiemsakke.*

L. rugosa *Thunb. vorm ook 'n* Polygonum *tipe kiemsak. Die sitoplasma van die* L. rugosa *kiemsak is baie digter saamgedruk, die vakuole is kleiner en die posisie van die poolkerne verskil van dié van* L. camara. *Dit is moontlik om* L. camara *van* L. rugosa *te onderskei slegs op grond van kiemsakmorfologie.*

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