

CYTOLOGICAL AND EMBRYOLOGICAL STUDIES IN THE GENUS *EUPHORBIA*: *EUPHORBIA EPITHYMOIDES* L.

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The female gametophyte of *E. epithymoides* conforms to the development in *Euphorbia dulcis*. However, the following types of embryo sacs also occur: 16-nucleate *Chrysanthemum parthenium* type, 12-nucleate *Chrysanthemum cinerariaefolium* type with the megaspore nuclei arranged as 1+(2)+1 or 1+1+2; and 4- and 8-nucleate *Plumbagella* type.

The following chromosome formula has been established:

$$z = 2n = 14 = 2M_1 + 2M_2 + 2M_3 + 2S_1^s + 2S_2 + 2S_3 + 2S_4.$$

INTRODUCTION

From a strictly taxonomic point of view there are many uncertainties about the delimitation of the genus *Euphorbia* and its species and subspecies.

There is no detailed cytological knowledge which would permit a clear understanding of the evolution of karyotype. Occurrence of the tetrasporic embryo sacs is known for *E. palustris*, *E. procera* and *E. dulcis*. Only in the latter species adventitious embryony is known, in connection not so much with the supposed hybrid origin of the species (Carano 1926) as with the polyploidy of some biotypes (Cesca 1961). The study of *E. epithymoides* promised to be interesting because of its systematic complexity including as it does several subspecific entities distributed over a large area, and because of the affinities it shows with the above-mentioned species.

MATERIAL AND METHODS

According to Fiori (1925/1929), in Italy *E. epithymoides* includes at least six subspecies. The plants studied by me belong to the 'B' group, i.e. var. *verrucosa* (Jacq.) (Lam.) (non L.) Fiori and var. *flavicomis* (DC.) Fiori. They are hairy plants with hard stems at the base; 20–50 cm high; umbels of five rays; capsules covered with short, uneven, cylindrical warts, red at maturity, cyathia with two glands, less frequently three, in some cases (usually cyathia without female flower) four to five. Differences noted in the samples of different localities will be the subject of subsequent publications.

Karpetschenko's fixative was used for preserving the cyathia picked from plants grown in their natural habitat on Mount Matanna (the Apuanian

Mountains) (June 1962 and May 1963), in the area of Mount Prato Fiorito (Lucca) (June 1964) and at Madonna della Neve near Givoletto (Turin) (July 1964). Additional material was collected, during 1963–1965, from plants brought from Mount Matanna and raised in pots (Figs. 1, 2).

The sections were stained with Heidenhain's haematoxylin. For karyological analysis the root tips were pre-treated with colchicine, fixed in acetic alcohol, hydrolysed, squashed and observed under a phase-contrast microscope without any staining.

OBSERVATIONS

Karyotype.—Seven bivalents were observed in the plants of Mount Prato Fiorito. A complete analysis of the karyotype was not possible, however, either in these or in those of Givoletto. Such analysis was done instead in samples coming from Mount Matanna.

The diploid chromosome complement (Figs. 5–9) consists of 14 chromosomes grouped in seven homologous pairs according to the formula:

$$z = 2n = 14 = 2M_1 + 2M_2 + 2M_3 + 2S_1^s + 2S_2 + 2S_3 + 2S_4$$

The first three pairs are of chromosomes of medium length, heterobrachial, with submedian centromere. The S_1^s pair, of the short type, with nearly median centromere, is distinguishable from all the others by the presence of intercalary pseudo-satellites well evident in the great majority of the chromosome plates. The other S pairs have nearly median centromere.

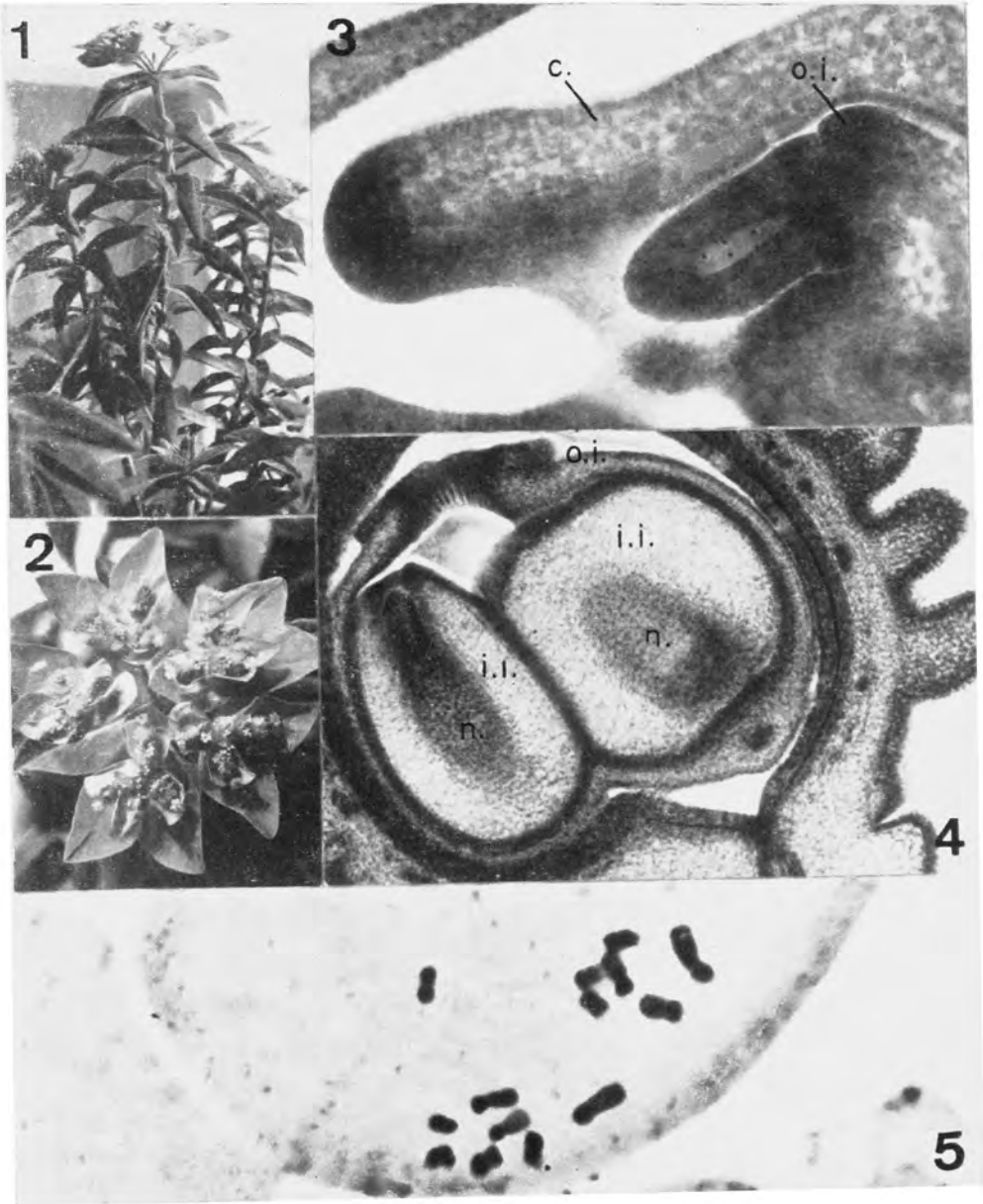
Male Gametophyte.—Meiosis takes place regularly and cytokinesis of microspores is of the simultaneous type. The division of the generative nucleus takes place while the pollen is still in the anther.

Female Gametophyte.—As has already been illustrated in other species of *Euphorbia* (see for example Kapil 1961), the ovule is crassinucellate and provided with two integuments. Two ovules enclosed in a common outer integument were also observed (Fig. 4).

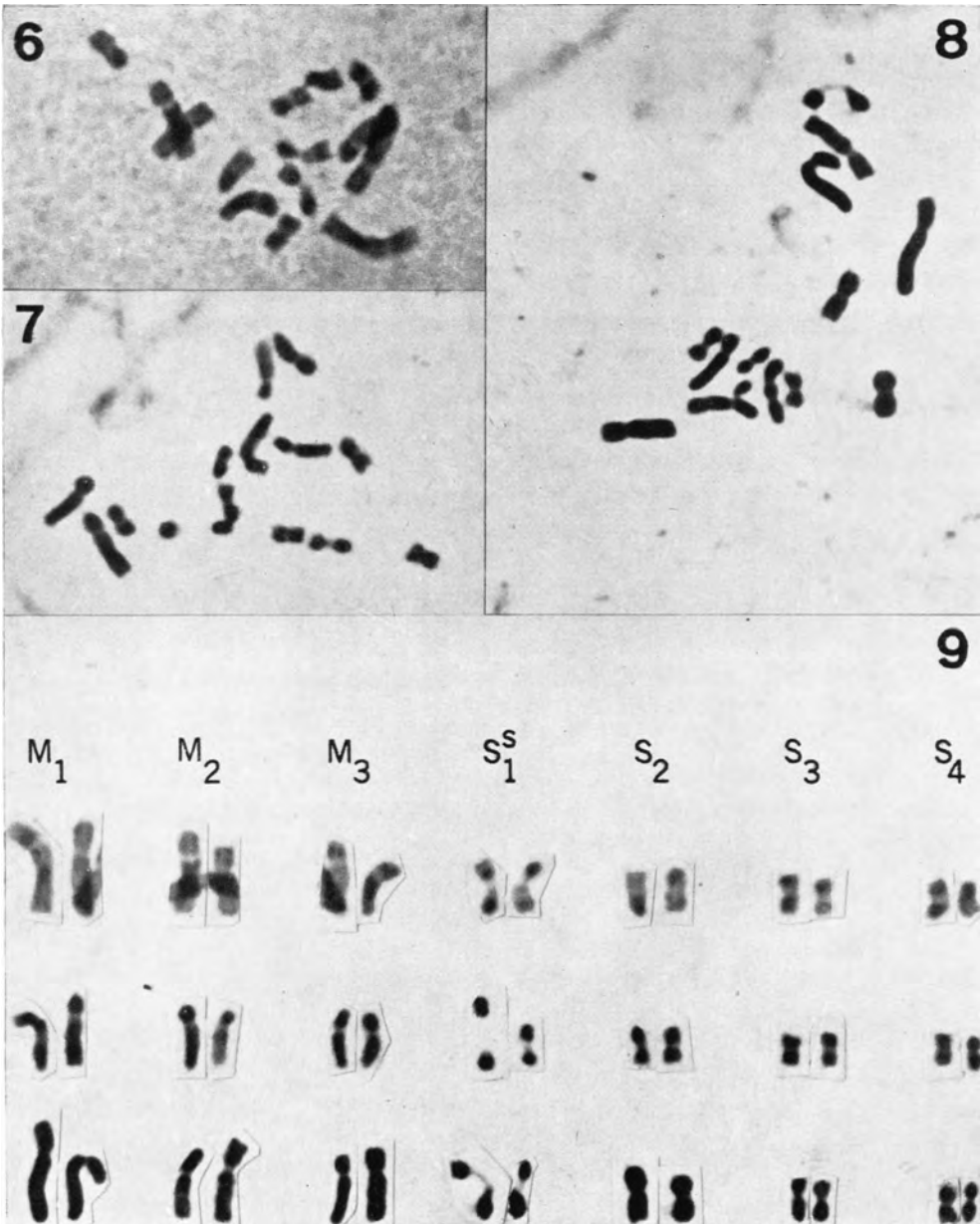
There is one, rarely two, megaspore mother cell. Meiosis occurs rather early while the carpels are still open and the ovule shows only the primordium of the outer integument (Fig. 3). The meiotic divisions are not followed by wall formation and give rise to a 4-nucleate coenomegaspore (Figs. 10, 11, 12, 28, 29, 30). Sometimes, at telophase of the second meiotic division, the two central chromosome groups become included in a common membrane, or these nuclei may fuse so that the coenomegaspore contains only three nuclei (Fig. 13).

The subsequent development of the coenomegaspore is very variable resulting in 4- to 16-nucleate embryo sacs with 1–3 micropylar and 1 to 11 antipodal nuclei.

Figs. 14–17 and 31–33 show the variations in the disposition of megaspore nuclei: 2+2; 1+1+2; 1+(2)+1; 1+3 (which is more frequent), or rarely

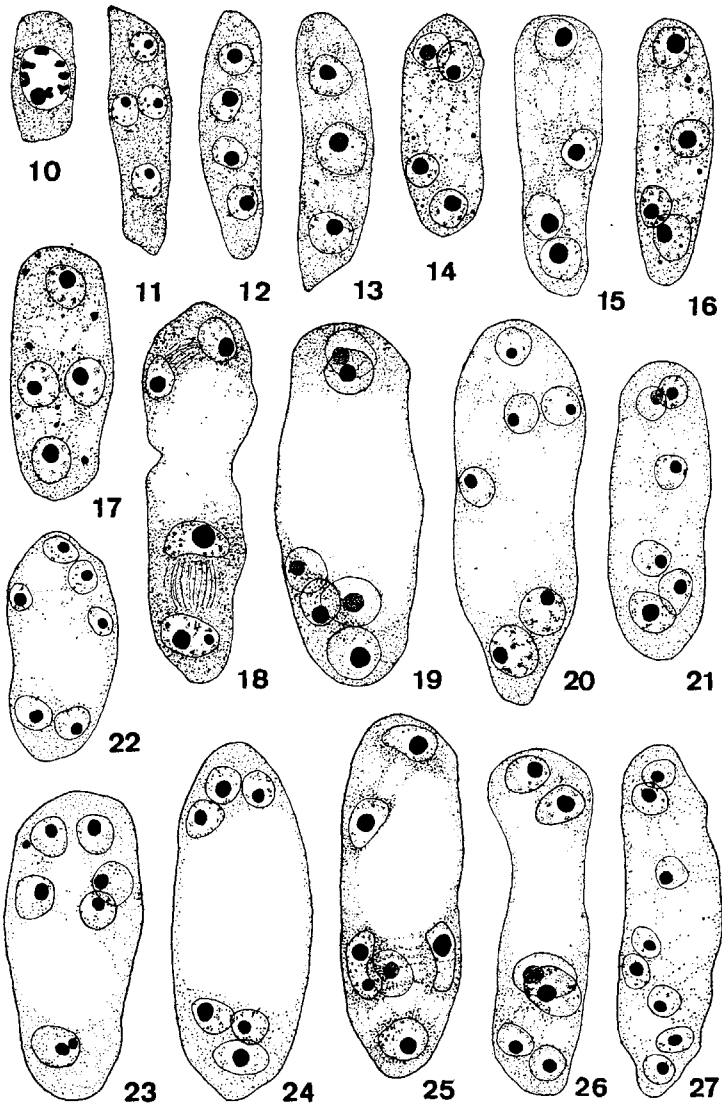


FIGS. 1-5. 1-2, *E. epithymoides* L. (plants of Mount Matanna). \times about 1/3. 3, ovary and ovule shortly after meiosis (*c.*, carpel; *o.i.*, outer integument). \times 303. 4, two ovules with a single outer integument (*i.i.*, inner integument; *n.*, nucellus). \times 91. 5, metaphase in root tip of a plant from Mount Matanna. \times 2800.



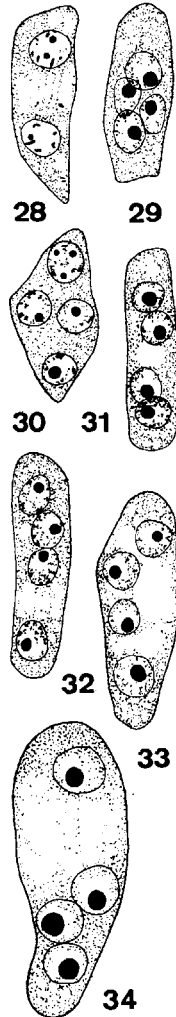
FIGS. 6-9. 6-8, metaphases in root tips of plants from Mount Matanna. $\times 2800$.
9, karyotype. $\times 2800$.

3+1. Movement of nuclei during later stages may permit the regularizing of uncommon dispositions, for example, embryo sacs referred to in the above figures may show the 1+3 condition (Fig. 34) at the next somatogenic division.



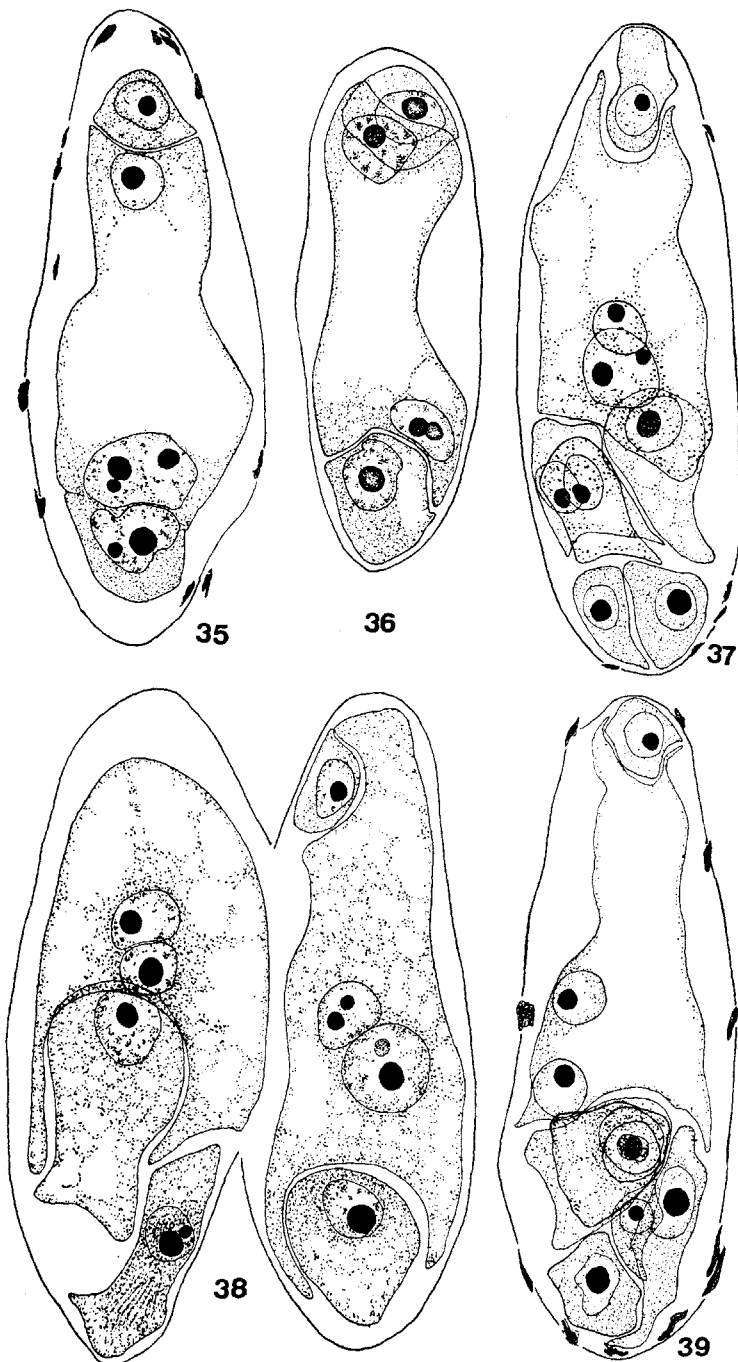
FIGS. 10-27. Material from Mount Pratoforito. 10, megaspore mother cell at diakinesis. 11 and 12, usual 4-nucleate coenomegaspores. 13, three-nucleate coenomegaspore due to fusion of the two central nuclei. 14-17, stages of vacuolation showing variable polarization of megaspore nuclei. 18, secondary 4-nucleate embryo sac due to a polarization 1+3 followed by fusion of chalazal nuclei. 19-24, variations in 6-nucleate embryo sacs as a result of a 1+1+2 arrangement of megaspore nuclei. 19, two haploid nuclei at the micropyle and four (two haploid and two diploid) at the chalazal end. 20 and 22, only two (diploid) chalazal nuclei. 21 and 24, successive stages of a polarization 3+3. 23, embryo sac like the preceding ones illustrated in 20 and 22, but with a single chalazal nucleus, the other ones having migrated to the micropylar end. 25 and 26, six-nucleate embryo sacs as a result of a 1+(2)+1 polarization. 27, eight-nucleate stage. All figures $\times 700$.

From a tri-nucleate coenomegaspore with the central diploid nucleus (Fig. 13), theoretically it is possible to obtain gametophytes with triploid chalazal nuclei as a result of a $1+2 (= 2n+n)$ polarization. The polarization, however, is not necessarily followed by nuclear fusion. The succeeding division (Table I: Somatogenesis, Division II) produces secondary 4-nucleate (Fig. 18), 6-nucleate (Figs. 19-26) or 8-nucleate (Fig. 27) embryo sacs.



FIGS. 28-34. Material from Mount Matanna. 28, end of the first meiotic division. 29 and 30, four-nucleate coenomegaspores. 31-33, vacuolation. 34, usual 1+3 arrangement of megaspore nuclei. All figures $\times 700$.

A further increase in the number of nuclei may be inhibited by the laying down of cell walls. Because of this, it has been possible to observe 4-nucleate embryo sacs of the *Plumbagella* type (Figs. 35, 36) and 8-nucleate embryo sacs



FIGS. 35-39. 35 and 36, *Plumbagella* 4-nucleate embryo sacs in material from Mount Matanna and from Mount Prato Fiorito respectively. 37-39, embryo sacs of the '*Plumbagella*' 8-nucleate type observed in plants from Mount Matanna only. 38, shows two successive sections of a very rare embryo sac with a micropylar cell (egg), two proendospermatic binucleate cells and three antipodal cells. All figures $\times 700$.

which also I consider to be of the same type (Figs. 37-39). Embryo sacs of 6-nucleate *Plumbagella* type, although theoretically possible and already described (Boyes 1939), were not observed. Similarly, wall formation has not been observed in 8-nucleate embryo sacs derived from a 2+2 disposition of megaspore nuclei. In Table I, the *Adoxa* type is, therefore, illustrated as only theoretically possible.

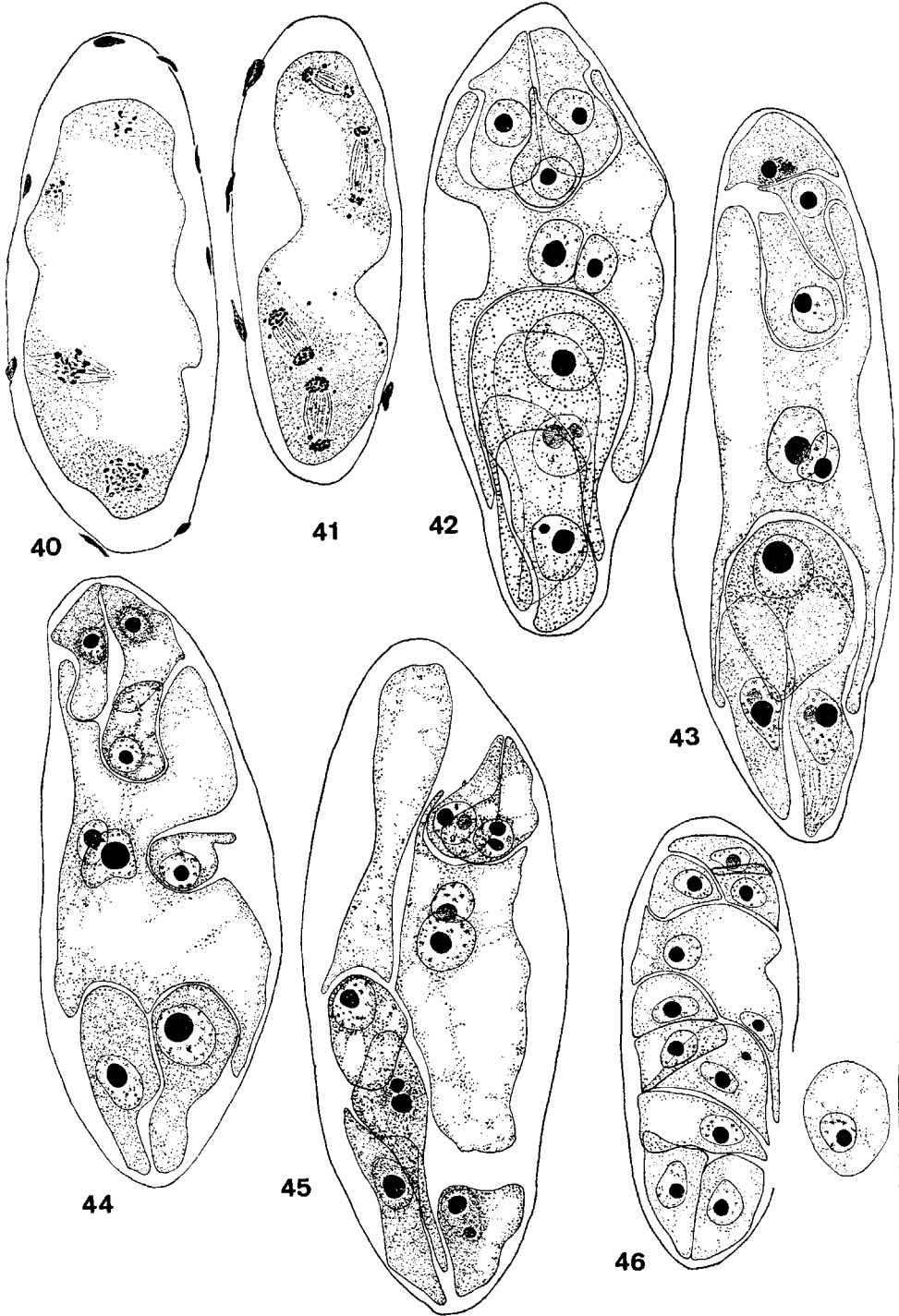
If the second somatogenic division is not followed by wall formation, the third and last division occurs normally and gives rise to embryo sacs belonging to different types (Figs. 40-48), i.e. 16-nucleate *Chrysanthemum parthenium* type (starting from the 8-nucleate stage); 12-nucleate *Chrysanthemum cinerariaefolium* 'A' type [starting from the 6-nucleate with 2 (haploid)+2(2n)+2 (haploid) nuclei], and 'B' type [starting from the 6-nucleate with 2 (haploid)+2 (haploid)+2(2n) nuclei]; 8-nucleate *Euphorbia dulcis* type [starting from the secondary 4-nucleate embryo sac].

In the material examined by me, the above-mentioned types of embryo sacs were observed with varying frequency. In all the plants, the most usual is the *Euphorbia dulcis* type. In the scant material from Givoletto, I usually observed this type, in addition to a few mature gametophytes with 16 or 12 cells and a single degenerated embryo sac with a micropylar cell (the egg) without any remnants of synergids. The plants from Mount Prato-fiorito showed 16-nucleate *Chrysanthemum parthenium* type (1 to 2 per cent of the classifiable embryo sacs), 12-nucleate *Chrysanthemum cinerariaefolium* type (about 9.5), 4-nucleate *Plumbagella* type (a single embryo sac out of more than 120), and *Euphorbia dulcis* type (89.5). In the material from Mount Matanna, I noticed about 97 per cent embryo sacs of the *Euphorbia dulcis* type, 2.4 per cent 4- and 8-nucleate *Plumbagella* type, and only one embryo sac with 12 or more cells.

The mature embryo sacs (Figs. 35-39, 42-48) have a more or less oval outline with very large antipodal cells. At least one of these is egg-like while the other antipodal cells, usually the chalazal ones of the 12- or 16-nucleate embryo sacs, simulate synergids and often show the 'filiform apparatus'. It is not rare to find gametophytes with large proendospermatic cell whose nuclei lie close to the cell wall on the side of the egg-like antipodal cell rather than on the side of the egg-cell as it is the usual case.

Two adult gametophytes (Figs. 44, 45), lying side by side, developed from two adjacent mother cells; and an anomalous 12-nucleate embryo sac showed 2-nucleate antipodal cells (Fig. 51). Probably, abnormal wall formation is a

FIGS. 40-46. 40 and 41, embryo sacs showing the second somatogenic division with triploid chalazal nuclei. 42-45, aspects of *Euphorbia dulcis* 8-nucleate embryo sacs. 44 and 45, successive sections of the same ovule containing two embryo sacs formed by development of two megaspore mother cells. 46, a young embryo sac of the *Chrysanthemum cinerariaefolium* type. All figures $\times 700$.



direct consequence of a disturbed polarity in the preceding stages (Figs. 49, 50).

The pollen tube reaches the embryo sac through the nucellar beak. After fertilization, the primary endosperm nucleus divides (Fig. 52) three or four times before the division of the zygote.

Despite their large size, the antipodal cells degenerate and disappear altogether during early development of the proembryo.

Often, in *E. epithymoides*, the first cyathia of the umbel (cyathia with 4/5 glands) bear male flowers only, or also a female flower destined to wither. Moreover, it is difficult to establish if a certain percentage of sterility, due to degeneration of embryo sacs or young embryos, affects principally the less usual type of embryo sacs. It is likely, though, to assume disturbances in the formation of endosperm and embryo in anomalous embryo sacs such as the two illustrated in Figs. 49 and 50.

DISCUSSION

Euphorbia verrucosa [*Tithymalus verrucosus* (L.) Rafin. according to Löve and Löve 1961] to which Shimoyama (1958) assigns the numbers $n = 7$ and $2n = 14$ belongs to the *E. epithymoides* L. complex (Fiori 1925/1929), and is probably the same plant I have studied. Further studies, with special attention to synonymy and correct classification, would be necessary to clarify the affinities between this taxon and *E. polychroma* Kerner [= *E. epithymoides* L.; *Tithymalus polychromus* (Kerner) Prokh.] having $2n = 16$ chromosomes (Perry 1943; Polya 1950; Shimoyama 1958; 'source: Germany') and 14 chromosomes (Shimoyama 1958; 'source: Hungary').

Useful comparisons are possible with karyotypes of *E. spinosa* L., *E. bivonae* Steud., and *E. acanthothamnus* Heldr. et Sart. These species have four M pairs and three S pairs of chromosomes. In *E. epithymoides* the M_4 pair is missing, substituted by the S_1^5 pair related to the one characterized by the same symbol in the species cited.

Tetrasporic embryo sacs are well known in the genus *Euphorbia*: *Penaea* type in *E. palustris* and *E. procera* (Modilewski 1909, 1910, 1911); and the *Euphorbia dulcis* type in this species (Carano 1926; Kapil 1961) and also in *E. bivonae*, *E. acanthothamnus* and *E. epithymoides* (Cesca 1966; present paper). The latter species differs from the others in the appearance and large size of antipodal cells.

FIGS. 47-52. Material from Mount Prato Fiorito. 47, a more advanced embryo sac of the *Chrysanthemum cinerariaefolium* type. 48, sixteen-nucleate embryo sac organized according to the *Chrysanthemum parthenium* type. 49, young anomalous 16-nucleate embryo sac totally divided in large cells, one of them being binucleate. 50, twelve-nucleate embryo sac with four micropylar cells and no binucleate proendospermatic cell. 51, part of an anomalous 12-celled embryo sac with two binucleate antipodal cells. 52, embryo sac showing degenerating synergids, zygote, first division of endosperm and three antipodal cells. All figures $\times 700$.

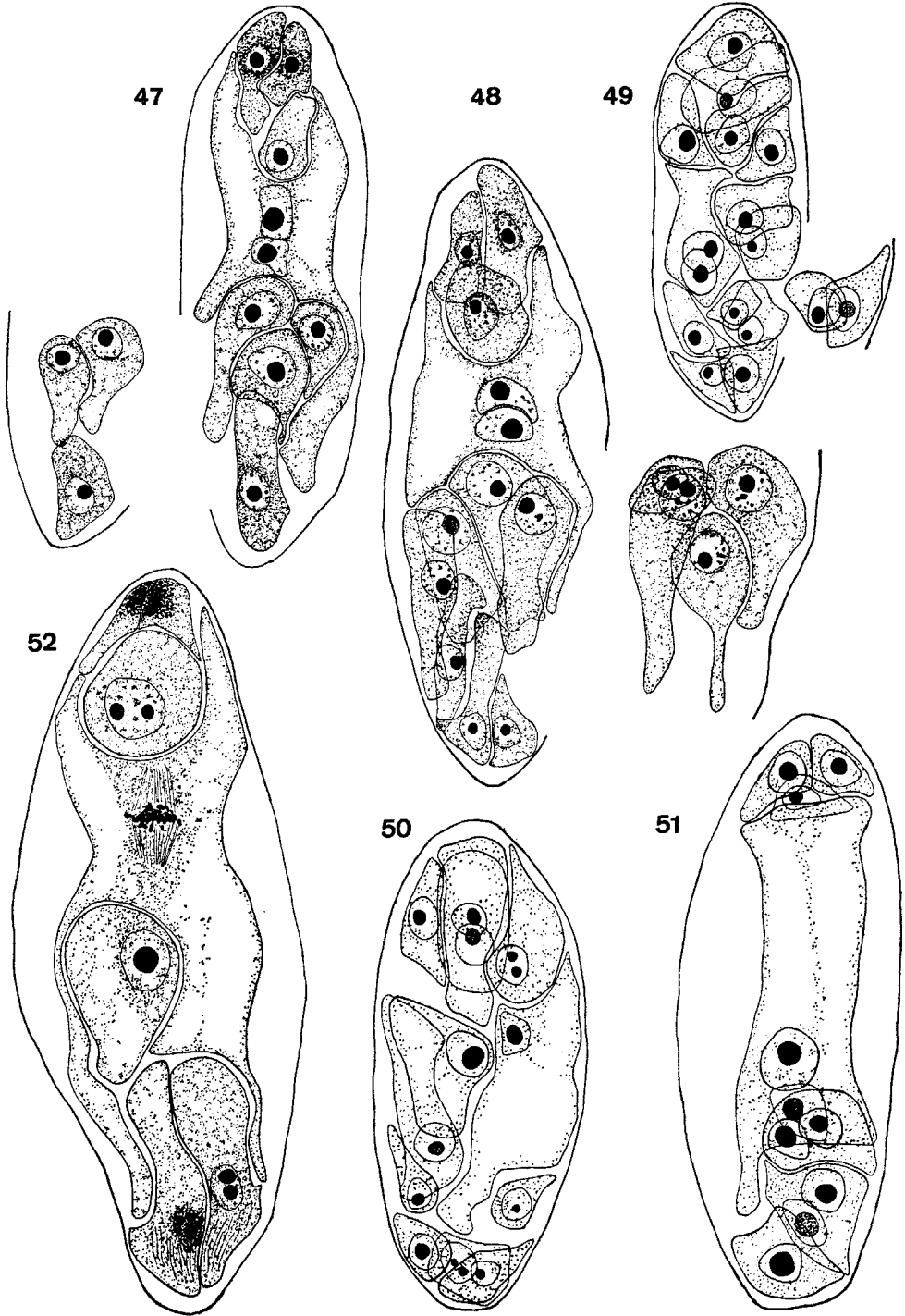
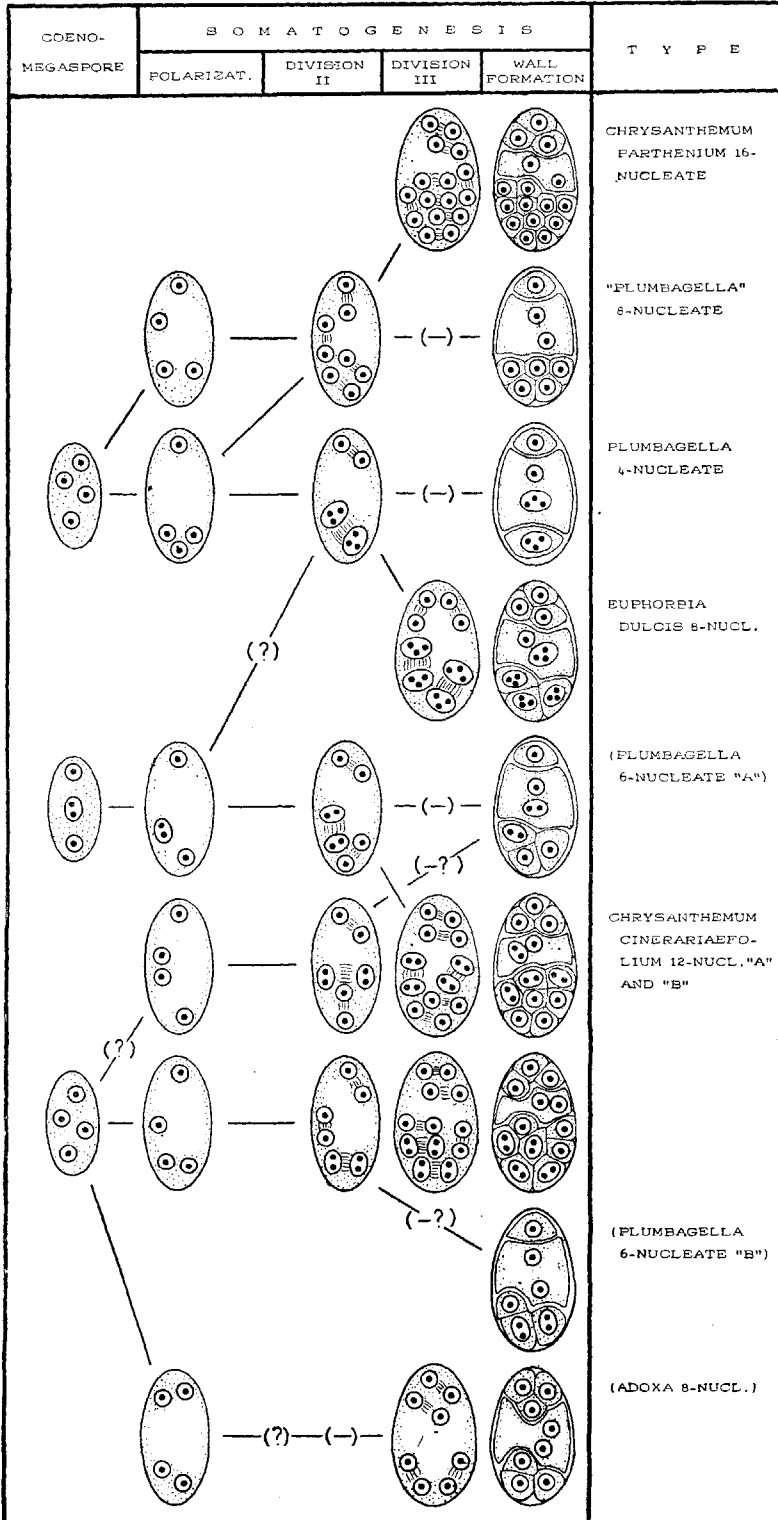


TABLE I

Diagram showing the tetrasporic types of embryo sac development (observed or—
between brackets—theoretically possible in *Euphorbia epithymoides*)



The '*Plumbagella*' type of 8-nucleate embryo sac with a single micropylar cell, that is *Plumbagella* type without the 'Bambacioni phenomenon', is a new finding. Such an 8-nucleate condition gives evidence in favour of the independence of the *Plumbagella* type from other more or less related types and, in particular, *E. dulcis* (= *Fritillaria*) type from which Boyes (1939) and later authors consider it derived.

The *Chrysanthemum parthenium* type is known for example in *Euphorbia dulcis*. In the same species stages have been observed which make one to presume the development of some embryo sacs according to the *Chrysanthemum cinerariaefolium* type, which is well ascertained in *E. bivonae* and *E. acanthothamnus*.

The method for the formation of embryo sacs conforming to the *Chrysanthemum cinerariaefolium* type is that of the polarization $1+(2)+1$ (Martinioli 1939) with fusion of the megaspore nuclei at a more or less early stage and their successive participation in the somatogenic divisions. To simplify matters, I have considered as a variation of the same type the one characterized by the letter 'B' in Table I, although, still having 12 cells at the mature stage, it differs in the type of polarization [$1+1+2$ rather than $1+(2)+1$], in a consequently later fusion of the nuclei and in the diverse degree of ploidy of the lower polar nucleus. Such modality has not even been observed in *Chrysanthemum cinerariaefolium* and represents a phase of transition towards the *E. dulcis* type.

Variations in embryo sac development in the same plant have not been very frequently reported. Among the most recent examples *Tamarix odessana* and *T. parviflora* (Hjelmqvist and Grazi 1964) must be mentioned, in which the *Chrysanthemum parthenium*, *Chrysanthemum cinerariaefolium*, *Adoxa*, *E. dulcis* and *Plumbagella* types have been observed with different percentages depending on temperature. In *E. epithymoides* the possible influence of the environment has not been experimentally studied. It is not excluded, however, that there exists some connection with the previously mentioned uncertainty in important morphological peculiarities (number of glands per cyathium), both facts being influenced by the same internal imbalance.

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