

APOMIXIS AND HEMIGAMY AS ONE OF ITS FORMS*

by M.P. SOLNTZEVA, *Laboratory of Plants Embryology, Department of Morphology, Komarov Botanical Institute, Academy of Sciences of the U.S.S.R., 2 Prof. Popov Street, Leningrad, 197 022, USSR*

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The phenomenon of apomixis is widely distributed in nature. Presently, apomixis has been established in over 290 genera.

Apomixis is very variable in its manifestation, ranging from aposporic parthenogenesis to adventive embryony.

In most cases apomixis is hereditary and is conditioned and controlled by a whole "constellation of genes" (Burton & Forbs, 1960). The hereditary mechanism of various forms of apomixis is still unknown.

Many cultivated plants such as citrus, dewberry, and mango show apomixis as the main form of reproduction. The breeding of these plants demonstrates that apomixis does not prevent but, rather, promotes more successful selection of new variations and forms with valuable economically important features. Such plants form the first group.

There is another group of plants which have only isolated elements of apomixis. According to Powers (1945) the Elements of apomixis are: (a) lack of reductional division; (b) capacity of egg cells to develop without fertilization; and (c) capacity of the embryo sac secondary nucleus to develop without fertilization and produce endosperm, etc. (Petrov, 1964). The selection of plants with these isolated elements of apomixis and their further crossing may result in the appearance of forms and varieties which reproduce exclusively through apomixis (Petrov, 1964).

The third group includes important cultivated plants like wheat, rye, tobacco, rice, potato, sorghum, tomato, and many others. The phenomenon of apomixis is spontaneous in these plants and haploids are quite common.

Navashin (1933) stated that it was possible to use apomictic haploids in selection and when the number of chromosomes is doubled homozygotic plants are formed. Such plants are especially valuable for breeders. Along with haploids

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FIG. 1. *Rudbeckia laciniata*. Hemigamy. (Abbreviations: O, ovule; S, synergids; sp, sperm; a, antipodal cell; p.e.n, primary endosperm nucleus.) A, Embryo sac during fertilization; B, Portion of the same embryo sac. One of the sperms is in the egg cell, the other one fuses with the central nucleus (1-2); C, Prophase of the egg cell nucleus and sperm nucleus: 1, The focus is at the sperm nucleus. 2, The focus is at the egg cell nucleus; D, Embryo sac in which a synchronous division of the egg cell and sperm is taking place. There are two egg cell-like antipodal cells; E, The section of the same embryo sac. The egg cell with metaphase of the sperm nucleus; F, Same enlarged view; G, Next section of the same embryo sac; metaphase of the egg cell division. →

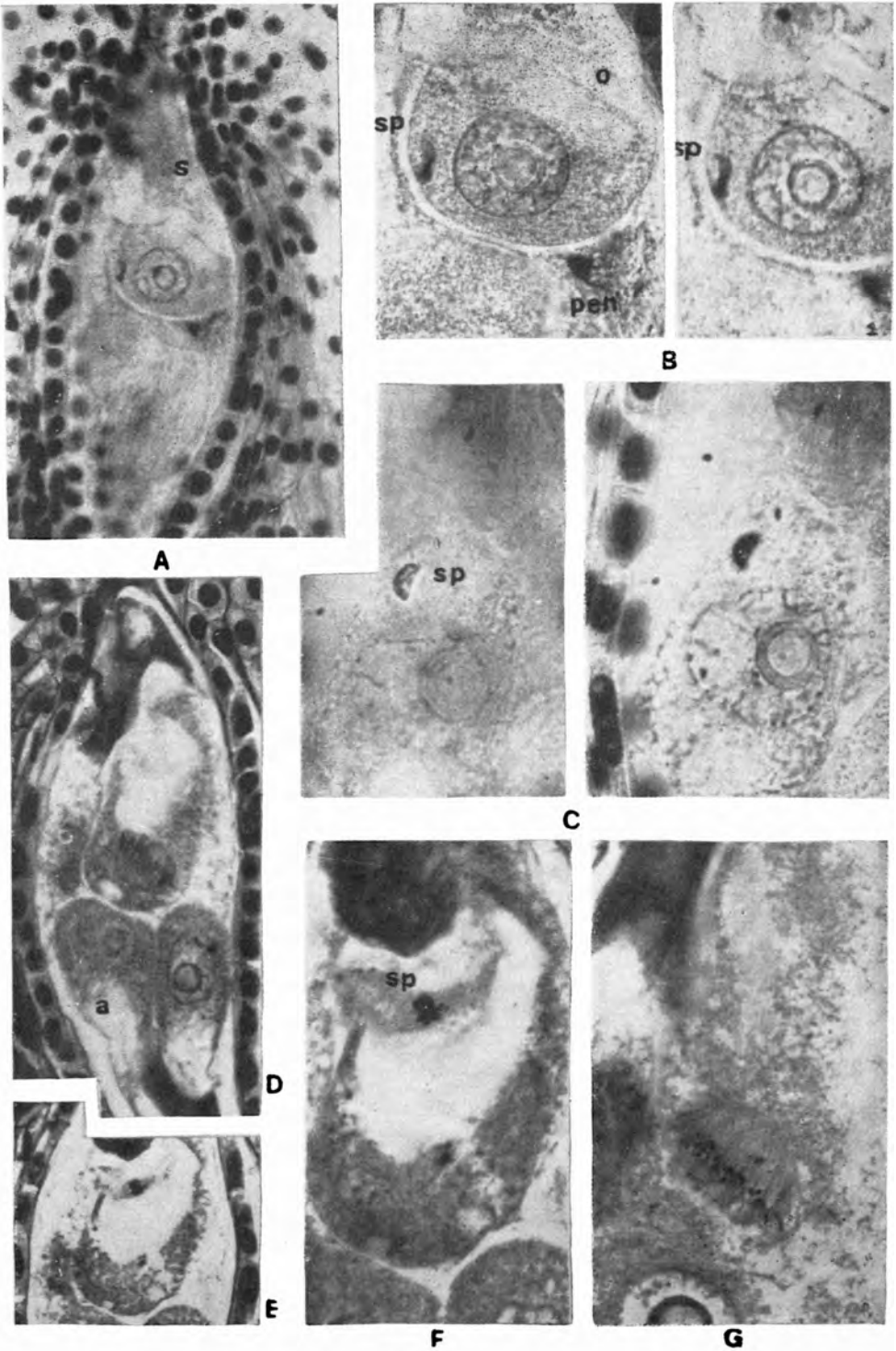


FIG. 1.

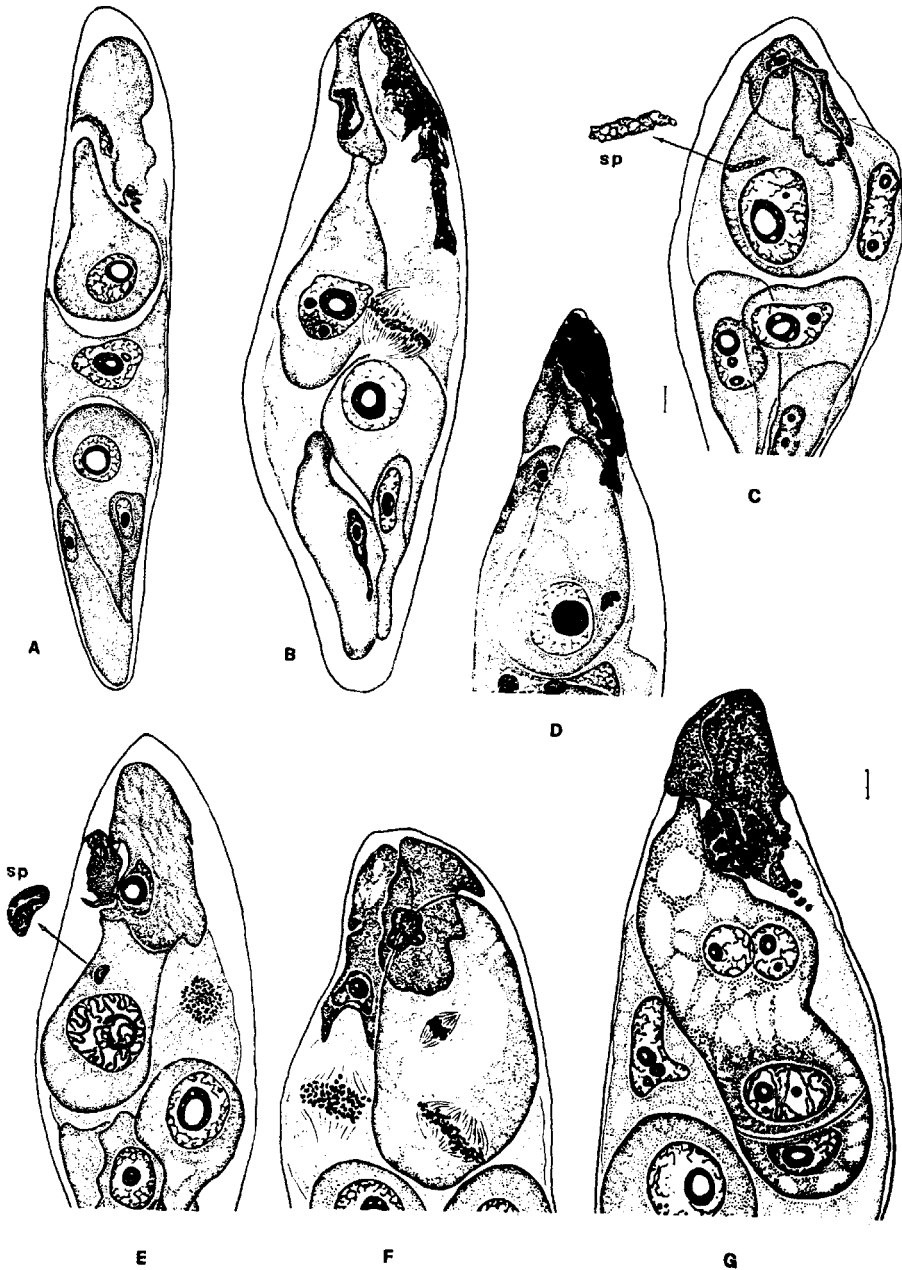


FIG. 2. *Rudbeckia laciniata*. Fertilization and hemigamy. *A*, Sperms passing through the synergid; *B*, The fusion of the sperm with the egg cell nucleus. The primary endosperm nucleus is in metaphase; *C*, The elongated sperm is in the egg cell; *D*, The sperm starts rounding off in the egg cell; *E*, The sperm and the egg cell nuclei are in prophase; the primary endosperm nucleus is in metaphase; *F*, Synchronous metaphase of division of the egg cell and sperm; *G*, Four-nucleate embryo with two large nuclei (egg cell derivatives), and two small ones (sperm derivatives).

apomictic diploids are also formed perhaps from the unfertilized haploid egg cells. This has been found in maize (Randolph, 1932; Yarnell & Hills, 1959; Gorcline & Thomas, 1964; and Annenkova & Sumanov, 1965); and in wheat (Tschermak-Seyengg, 1951; Kandelaki, 1969). Kandelaki (1969) considers that the doubling of chromosomes takes place in the old egg cell of wheat by endomitosis. These diploid embryos of pure female type (diploid parthenogenesis) develop when stimulated by pollination, but without sperm penetration. (It was proved by cytophotometric method.)

Absolutely new forms such as *Triticale* were created by Levitsky and Benetskaya (1931). This plant had complete chromosome sets of wheat and rye ($42+14=56$). According to Levitsky and Benetskaya (1931) and Lebedev (1935), the formation of these amphidiploids took place as a result of the development of unfertilized unreduced egg cells, accompanied by the doubling of their chromosome sets at the first cell-division. A similar phenomenon has also been observed in other plant species, for example, sorghum. However, embryological data elucidating the phenomenon of duplication of chromosome sets are not available. Thus, probable reduplication of chromosomes remains a theoretical possibility. Apomixis has another very important biological feature. It fixes heterosis or hybrid vigour of plants. It is, however, very difficult and expensive to maintain. Using apomixis it could be possible to obtain fixation of heterosis. This idea of heterosis fixation using apomicts was first proposed by Navashin (1933) and later by Karpechenko (1935). Chase (1949) and his successors successfully demonstrated this in practice.

In maize isolated elements of apomixis occur, and haploid embryos are frequently formed. It should be said that such haploids appear with a very low frequency (1 : 1000), but the lines with 3% haploids have already been found (Coe, 1959). Moreover, maize too has unreduced gametes. Formation of diploid egg cells may partly take place at the expense of additional aposporic embryo sacs (Chebotaru, 1965) and, sometimes due to the duplication of haploid set in egg cell as is described for sugarcane (Narayanaswamy, 1940), and wheat (Kandelaki, 1969). As a result of formation of unreduced gametes by apomixis, diploids and tetraploids are formed (frequency 6.0 to 0.04 : 1000). It is these apomictic forms that are capable of heterosis fixation.

Certain phenomena of apomixis are well known to plant breeders, for example, parthenogenesis and adventive embryony, have been applied in agriculture. Some phenomena have been discovered recently, and have not been sufficiently studied, e.g. hemigamy (semigamy).

The term 'semigamy' introduced by Battaglia (1945) is rather complicated (from the linguistic point of view), and is made up of both Latin and Greek roots. When translated into Greek, it sounds as 'hemigamy'. I, therefore, prefer to use the Greek form.

Hemigamy is an incomplete fusion of gametes during fertilization when the sperm nucleus penetrates the egg but does not fuse with its nucleus, preserving its viability like the egg cell nucleus and dividing, later on, independently.

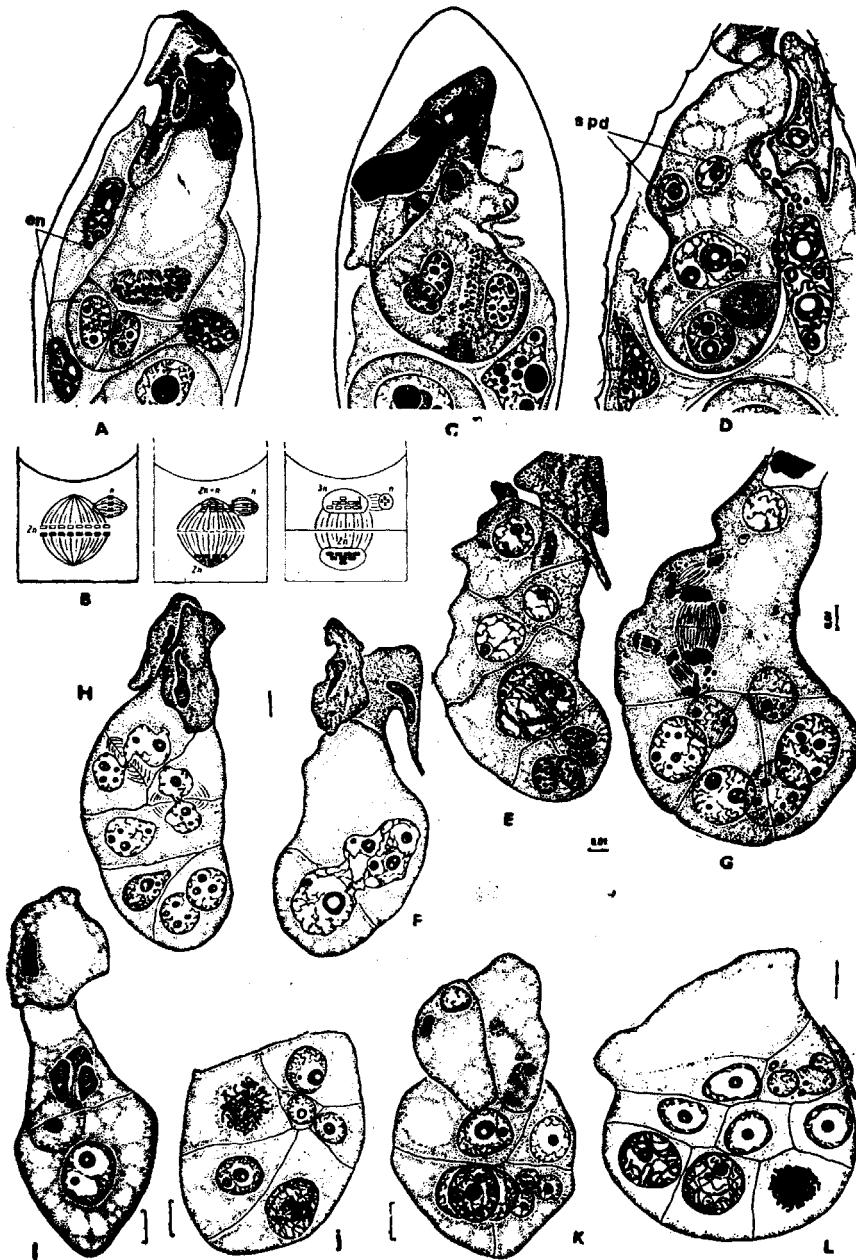


FIG. 3. *Rudbeckia laciniata* and *Zephyranthes macrosiphon*. (I-L). Embryo. (Abbreviations (for Fig. 3 and others): *sp.d.*, sperm nucleus derivatives; *e.d.*, egg cell nucleus derivatives; *t.n.*, triploid nucleus; *e.n.*, endosperm nucleus; *r.n.*, restitutional nucleus.) *A*, Synchronous division (metaphase) of the sperm nucleus and one of the derivatives of the egg cell nucleus is taking place in one of the cells of the embryo; *B*, Scheme of the egg cell nuclear fusion during the division. Note the formation of haploid (n), diploid ($2n$), and triploid ($3n$) (continued on facing page)

At present hemigamy is known in several families, both in the dicotyledons and monocotyledons. There are 6 species of *Rudbeckia* in family Asteraceae, 3 species of *Zephyranthes* and 2 of *Cooperia* in Amaryllidaceae, and also *Arabidopsis thaliana* in Brassicaceae (under experimental conditions). Genetic studies prove the occurrence of this phenomenon in the genus *Gossypium* (Malvaceae). Unfortunately, this has not been confirmed by embryological studies.

The phenomenon in question is of a specific character in different plants, and can take on different forms in one and the same plant. In *Rudbeckia laciniata* normal fertilization takes place in a certain number of cases but, in some cases, severe abnormalities are observed. Under the normal course the pollen tube enters the embryo sac through one of the synergids (Fig. 1a, 2a). The sperms enter the embryo sac and have been observed at various distances from the female nuclei. One sperm comes into contact with the central nucleus (Fig. 1, a, b), and fuses with it. The second sperm implants itself into the egg cell, and it was possible to observe normal fertilization according to premitotic type (Fig. 2 b).

Such a course of sexual process is observed rarely. Even in the ovules of the same inflorescence of the same plant, quite another process has been observed. The abnormality of fertilization takes place in the egg cell after the sperm penetrates its cytoplasm (Figs. 1 & 2). The sperm does not fuse with the nucleus of the egg cell but remains at a certain distance from it (Fig 1a, b & 2c), and gradually rounds off (Fig. 1 c & 2 c, d). Then the egg cell nucleus and the sperm nucleus divide simultaneously. (Fig. 1 d-g & 2f). In (Fig. 1, c-d) one can see that the nucleus of both egg cell and the sperm nucleus is in prophase. Metaphase of the sperm and the egg nucleus take place simultaneously (Fig 1 d-g, & 2f). As a result of autonomous division of these nuclei a 4-nucleate embryo is formed (Fig. 2 g). Two of the nuclei are the derivatives of egg cell (large nuclei), and two others are derivatives of the sperm (small ones). Previous studies on the development of female and male gametophytes (Solntzeva, 1973 a, b) showed that embryo sac nuclei are unreduced and diploid (as a result of formation of restitutional nuclei at meiosis I). The sperm nuclei are mainly haploid, though often they are hyperhaploid as a result of meiotic abnormalities.

In *Rudbeckia laciniata* walls are formed between the female nuclei but, in most cases, walls are not laid down between the male, or between the male and female nuclei (Fig. 3 d). Sometimes, walls develop between the male nuclei (Fig. 4 a, b). This probably depends on the condition and arrangement of the nuclei

nuclei; C, The fusion of one of the derivatives of sperm has taken place with the derivative nucleus of the egg cell; D, Further division results in formation of two haploid sperm derivative nuclei, two diploid egg cell derivative nuclei; the triploid nucleus remains undivided; E, The large triploid nucleus is in the central part of embryo; F, The anomalous division of triploid nucleus of the zygote results in formation of a restitutional haploid nucleus, G, Synchronous division of the derivative sperm nucleus, and of the female nucleus in one cell of the embryo; H, The formation of restitutional sperm nuclei during their second division; I, The formation of cell wall between the sperm derivatives; J, In the proembryo the sperm derivative nuclei are small; K, The formation of the restitutional male nucleus in one of the cells of proembryo; L, The micro-and restitutional nuclei formation in a proembryonal cell.

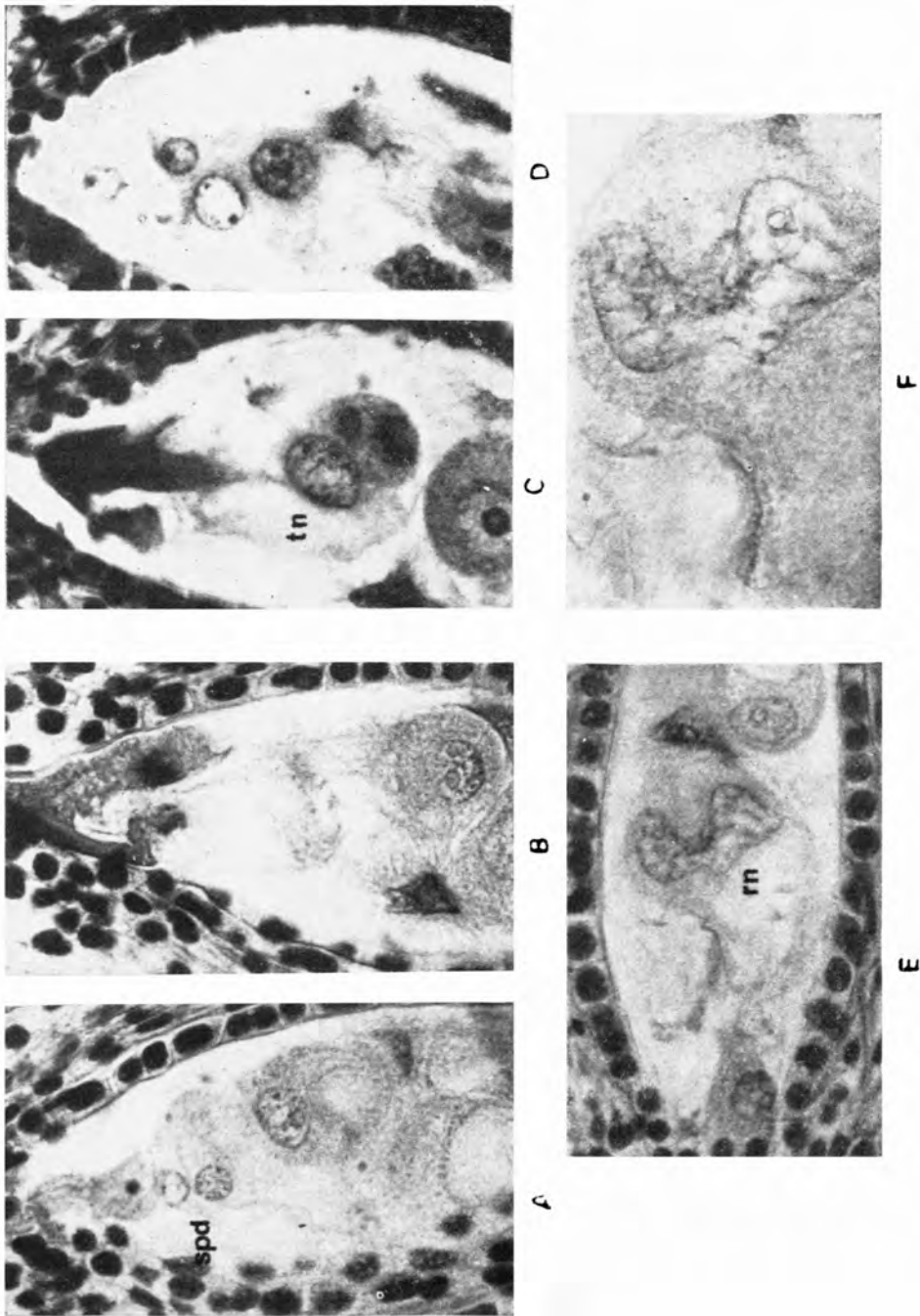


FIG. 4. *Radbeckia laciniata*. Proembryo. A & B, Two successive sections of proembryo with two large female nuclei (2n), and two small male nuclei (n). There is a wall between the sperm derivatives; C & D, Two successive sections of the proembryo with different ploids of nuclei, the large triploid nucleus is in the central part of proembryo; E, Restititional triploid nucleus appears during the first division of zygote nucleus; F, Restititional nucleus.

within the egg cell. If male and female nuclei are situated close to each other in one and the same cell (Fig. 3a, c), then these nuclei can fuse. In this case, fusion occurs during mitosis and triploid nuclei are formed (Fig. 3a-c). Such nuclei are very large, and do not divide for a long time (Fig. 3d, e, & Fig. 4, c). Afterwards they divide and show many abnormalities (Fig. 3 g), like chromosome bridges, or restitution nuclei (Fig. 4e, f). In the latter case the chromosomes become doubled and form hexaploid nuclei. Sometimes, during fertilization the diploid egg nucleus fuses with the haploid sperm nucleus. This triploid nucleus does not divide for a long time. When it does divide a hexaploid restitution nucleus is formed (Fig. 3 f). Afterwards this nucleus divides normally.

The male nuclei usually divide regularly only once, seldom twice. The subsequent divisions are irregular with the formation of chromosome bridges and diploid restitution nuclei (Fig. 3 f).

The same process has been observed in *Zephyranthes macrosiphon*, which we studied together with L.I. Vorsobina. In this taxon the sperm nucleus penetrates into the egg cell (Fig. 5 a-c) but does not fuse with the egg nucleus. In the egg cell, the egg nucleus divides first followed by the division of the sperm nucleus (Fig. 5 d). During the first division a wall is formed, walls are also laid down between the derivatives of sperm and egg nuclei (Fig. 5 d, e, & Fig. 3 i). Thus, a 4-nucleate proembryo is formed (Fig. 5h & i) with smaller daughter nuclei derived from the sperm (Fig. 3 i). The second division of the sperm usually takes place without any wall formation (Fig. 3 k). After the second division of sperm nucleus there are some abnormalities during mitosis resulting in micronuclei, aneuploid and restitutional nuclei (Fig. 3 k & l). The same behaviour is observed in *Zephyranthes candida*, but is rare.

Thus, in *Zephyranthes* we observed the process of duplication of male nuclei by restitution division. One can also see such a phenomena in the illustrations of Battaglia (1947), in *Rudbeckia laciniata*. Gerlach-Cruse (1970) pointed out that in *Arabidopsis thaliana* the derivatives of haploid sperm can fuse with each other, when they are in the same cell. s, the derivatives of sperm also become diploid.

Hemigamy in various taxa can be classified into three main groups:

1. On penetration into the egg cell, the sperm nucleus does not divide but remains active and, soon, isolates itself by a wall from other parts of the embryo.
2. The sperm nucleus divides in the egg cell, accompanied by a cell wall.
3. The sperm nucleus divides in the egg cell, and the division is not accompanied by formation of cell walls.

The first type was observed by Coe (1953) in *Cooperia drumondii*, when, on penetration into the egg cell, the sperm did divide but always isolated itself by a cell wall and formed the basal cell of suspensor.

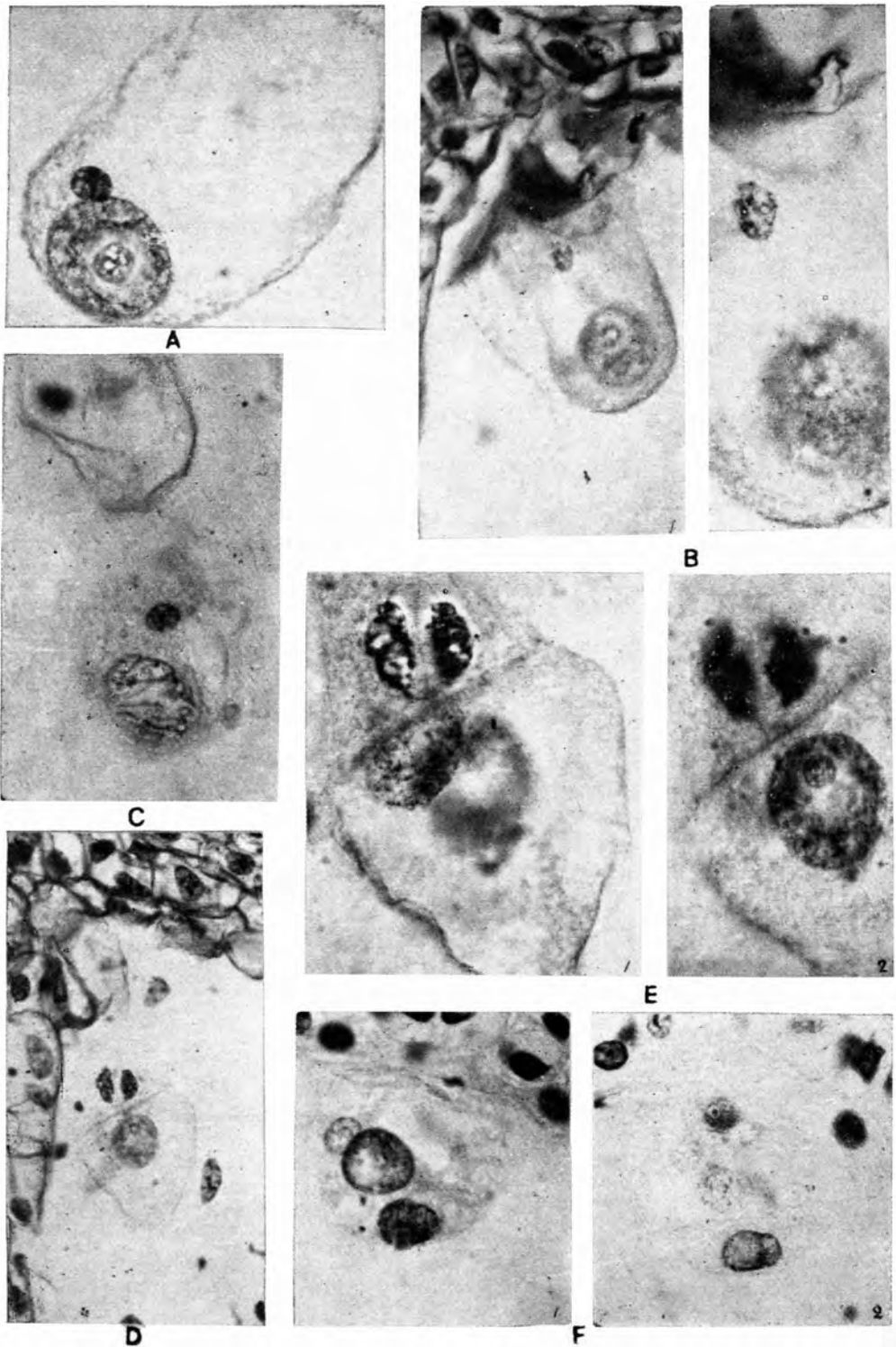


FIG. 5.

The second case when sperm nuclei divided and formed cell walls, was observed in a number of taxa of the family Amaryllidaceae, with some variations:

1. The sperm nucleus divides only once, and the division is accompanied by formation of walls both between the male nuclei and between the male and female nuclei. It has been observed in *Cooperia pedunculata*.
2. The sperm nucleus undergoes several divisions. During the first division it isolates itself from other cells by a cell wall; then its derivatives divide several times with accompanying wall formation (in *Cooperia pedunculata* the male nucleus divides twice), or without any wall formation.

Of course, data on the sperm contact and their derivatives in the embryo have not been completely studied but even then, one can trace the tendency to diploidization of male nuclei leading to the sufficiently long viability in embryo.

This hemigamous embryo is chimeric, mosaic, with nuclei of different ploidy and heredity: diploid maternal nuclei, diploid paternal, and hexaploid nuclei with combined heredity. The chimeric nature of embryos, which appears at early stages of proembryo development, may be preserved in the adult plant and affect the progeny.

The genetic study of doubled haploids in one variety of cotton by Turcotte and Feaster (1967, 1969, 1973) showed the formation of viable chimeric plants. The doubled haploid variety with dark-green leaves and oily glandules was pollinated with the pollen of the variety with light-green leaves and without oily glandules. This resulted in the formation of chimeric plants—some part of which had dark-green tissue and some were light-green (Fig. 6 A). Some plants had oily glandules on the stem, while others did not. One could observe sectors of chimeric tissues. Chimerism remains in generative organs as well, that is, in the flowers and the ovaries. In some of the plants both maternal and paternal flowers were observed (Fig. 6 B)

Tissue sectors had different ploidy: they were either haploid, or tetraploid. The authors consider that the presence of hemigamic lines eliminates obstacles in obtaining haploids of cotton which would be sufficient for production of homozygous lines. Hemigamy does not depend on the mode of pollination.

In *Rudbeckia laciniata* the number of cases of hemigamy and fertilization, was counted—both in cases of seed reproduction and in the clones, under different conditions of pollination: namely, self-pollination, cross-pollination, and free pollination. From the results obtained, it became clear that hemigamy occurs up to about 40% of all cases of pollination, while fertilization 20-25%. In 10-15%

FIG. 5. *Zephyranthes macrosiphon*. Hemigamy. A, The sperm nucleus is close to the egg cell nucleus. B, The degenerating sperm nucleus is at a distance from the egg cell nucleus (1). Enlarged view (2). C, The prophase of the egg cell nucleus, and the interphase of sperm nucleus. D, The embryo sac with 4-nucleate proembryo. Enlarged view (1-2). The cell wall has appeared between the sperm derivatives. E, Two successive sections of 4-nucleate proembryo (1-2); the small sperm derivative nuclei are in the micropylar part of proembryo.

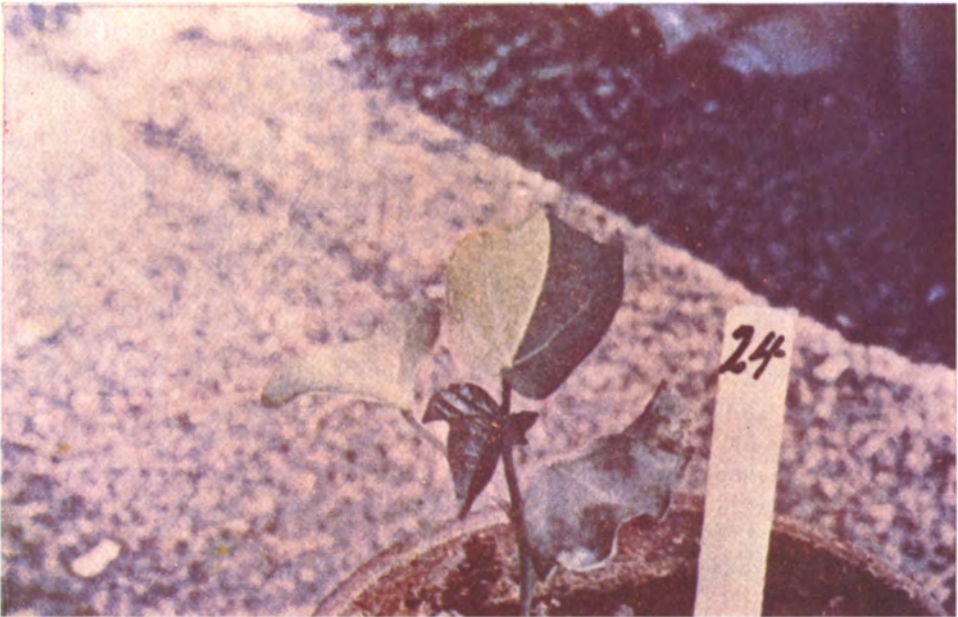


FIG. 6, *A. Gossypium barbadense* (according to Turcotte & Feaster, 1969). Seedlings with leaves of a himeric colour.

cases there are abnormalities of embryo sac development which result in the formation of uni-polar, 4-nucleate embryo sacs without the egg cell. In these embryo sacs the embryo is not formed. These data show that the hemigamy does not depend on the mode of pollination. Rather, it depends on some inner causes and to my mind, on incompatibility of male and female nuclei because of their different ploidy.

Comparing all the known cases of hemigamy with one another, and with other similar cases observable in a number of plant phenomena, for example, by interspecific hybridization, we conclude that hemigamy is a form of incompatibility of male and female nuclei, of the incompatibility of their chromosome sets because of different ploidy.

The discovery of chromosome set duplication during the very first division of triploid zygote in *R. laciniata* (when diploid egg cell nucleus is fertilized by haploid sperm), and also diploidization of haploid sperm by the formation of restitutional nuclei deserves special attention. Obviously, chromosome set duplications in haploid egg cells in maize, wheat, rice, sorghum, etc., can take place in exactly this way, i.e. by way of forming restitutional nuclei on account of faulty distribution of chromosomes during the first division of egg cell nucleus.

The study of such phenomenon as hemigamy, demonstrates not only one of the possible ways of polyploid tissue formation in the organism, but also indicates that this form of apomixis can result in the formation of specific polyploid series, and polyploid complex in populations.

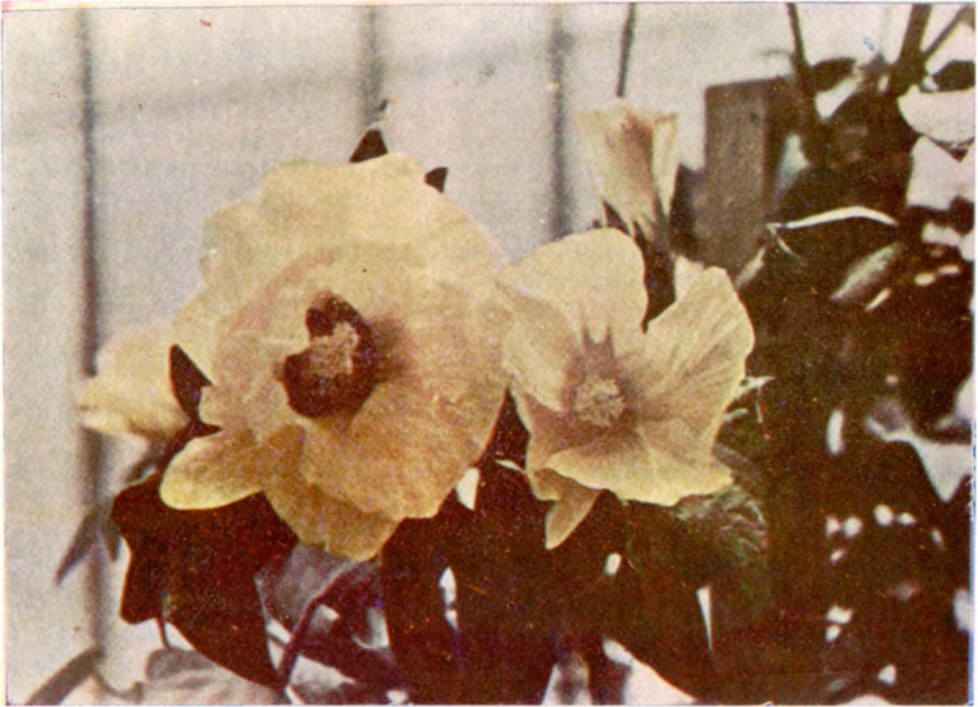


FIG. 6., *B. Gossypium barbadense* (according to Turcotte & Feaster, 1969). The maternal and paternal flowers appear on the same portion.

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