Theor Appl Genet (1992) 83:549–556

THEORETICAL AND APPLIED GENETICS

Expression of *ogu* cytoplasmic male sterility in cybrids of *Brassica napus*

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Received July 14, 1991; Accepted July 26, 1991
Communicated by H. F. Linskens

Summary. A light and electron microscopic investigation revealed that *ogu* cytoplasmic male sterility (CMS) in cybrids of *Brassica napus* is primarily a deficiency of the tapetum and clearly time and site specific. Three patterns of *ogu* CMS were found, and specific conclusions drawn. First, the partially male fertile cybrid 23 was highly variable. It sometimes produced heterogeneous stamens with an endothecium formed exclusively around the fertile locules, thus delineating each microsporangium as a functional unit. The second type, including cybrids 27, 58 and 85, on the contrary, was stable and completely male sterile. In the four locules of normal length, microspores were observed to die at the vacuolate polarized stage while the tapetum disappeared prematurely through excessive vacuolization by the end of meiosis followed by a rapid autolysis during the tetrad or early free microspore stage. The subepidermal layer of the locule wall failed to form characteristic thickenings. The male-sterile stamens were completely indehiscent. At the time of anthesis they contained only collapsed empty exines adhering to each other. These cybrids, 27, 58 and 85, were closest to the *ogu* CMS trait of radish and seemed to be the best suited for further use in plant breeding. The third pattern was found in cybrids 77 and 118, which besides showing abortion of the microsporangia also showed a feminization of the stamens. We suggest that this feminization might be due to an alloplasmic situation associating *Brassica napus* nuclear genes with the mitochondrial DNA of radish.

Key words: Cytology LM - SEM – TEM – Cybrid – Rapeseed – Radish – Cytoplasmic male sterility

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Introduction

Originally found in radish (*Raphanus sativus*) by Ogura (1968), the male-sterility-inducing *ogu* cytoplasm has subsequently been transferred to *Brassica oleracea* and then to *Brassica napus* (Bannerot et al. 1974).

This *ogu* cytoplasmic male sterility (CMS) was highly stable (Bartkowiak – Broda et al. 1979), but progeny plants showed a chlorophyll deficiency and low nectar production (Rousselle 1982). These two defects have been corrected by protoplast fusion (Pelletier et al. 1983), and male-sterile, chlorophyllous cybrids with well-developed nectaries are now available. The cybrids' cytoplasms can be distinguished from each other and from the *ogu* cytoplasm by their chloroplastic and mitochondrial genomes (Pelletier et al. 1983; Chetrit et al. 1985; Vedel et al. 1986). Thus different cytoplasms are now available in this CMS system.

By means of light and electron microscopy on paraffin-embedded stamens from the male-sterile radish he had just discovered, Ogura (1968) noticed that pollen degeneration occurred suddenly at the microspore stage and that this degeneration seemed to have some relation with the early collapse of tapetal tissue. Bartkowiak-Broda et al. (1979) also used this technique to investigate the cytology of sterile stamens of *Brassica napus* with *ogu* CMS. They found degeneration of the tapetum and the microspores after the tetrad stage, but their study was not very detailed. Some meiotic aberrations encountered in the flower buds were not expected to be the origin of the CMS, but rather a result of successive crosses.

Polowick and Sawhney (1991 a) used light and electron microscopy after aldehyde fixation and resin embedding, which is a more precise technique, for studying the influence of temperature on microsporogenesis in one *ogu* CMS line of *B. napus* in comparison with a normal line. The most prominent effect of temperature was the production of carpelloid stamens at the lowest temperature regime (18 °C/15 °C day/night) or bisexuate flower parts at the intermediate temperature regimes (23 °C/18 °C). From their cytologic study they concluded that there was an extensive variation in the abnormalities affecting microsporogenesis in *ogu* CMS of *B. napus* under their experimental conditions.

Cybrids of *B. napus* provide a new material both for plant breeding and research in genetics. Our cytological study on six cybrids and their comparison with the *ogu* CMS line from which these cybrids were derived and also a comparison of our data with those of Ogura (1968), Bartkowiak-Broda et al. (1979) and Polowick and Sawhney (1991 a) led us to make a retrospective survey on the expression of this CMS in *Cruciferae* over a period of two decades. The transfer of the CMS from the genus *Raphanus* to the genus *Brassica* probably created an alloplasmic situation. This will be discussed along with the process and consequences of cybrid formation in rapeseed.

Materials and methods

Plant materials

The male-sterile *B. napus* lines studied were one *ogu* line with *ogu* cytoplasm and six cybrids. These cybrids have been described by Pelletier et al. (1983). Cybrid 77 has *B. campestris* triazine-resistant chloroplasts and *R. sativus* mitochondria. Cybrids 23, 27, 58, 85 and 118 have *B. napus* chloroplasts and *B. napus*/*R. sativus* recombined mitochondria. All except cybrid 23 give homogeneous male-sterile progeny. Cybrid 23 is male-sterile except for one semi-sterile branch and gives rise to distinct progeny. The male-sterile part of the plant has completely male-sterile progeny 23-2. The semi-sterile part of the plant produced two progeny: (1) 23-3, which segregated in male-sterile and partially male-sterile plants and (2) 23-5, with only partially male-sterile plants.

The plants studied were grown in the field and in the greenhouse in 1986, 1987 and 1988.

For comparison purposes, the male-fertile *B. napus* lines 'Jet neuf' and 'Bienvenu' were also studied.

Light and electron microscopy

For rapid examination of the androecium and comparison of the locule content in male-fertile lines versus the male-sterile ones the stamens were mounted on a microscope slide in a drop of Alexander's stain (1969). Some of these whole mounts were first covered with a cover slide and then crushed with a needle during the experiments in order to distinguish the specific contnets of each locule; others were kept overnight at 30 °C prior to observation.

For electron microscopy (TEM) whole or sliced anthers at different stages of development were fixed in glutaraldehyde (2.5% w/v) for 14 h at 4 °C and post-fixed in osmium tetroxide (1% w/v) for 2 h (both in 0.1 *M* cacodylate buffer pH 7.2). After dehydration through an ethanol series and propylene oxide, the samples were embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope operating at 80 kV. Semi-thin sections (1 μm thick) from each sample were stained with toluidine blue and mounted in resin for light microscopy.

For scanning electron microscopy (SEM), both male-fertile and male-sterile flowers devoid of sepals and petals were fixed, dehydrated by the critical point method and then observed after gold sputtering.

Results

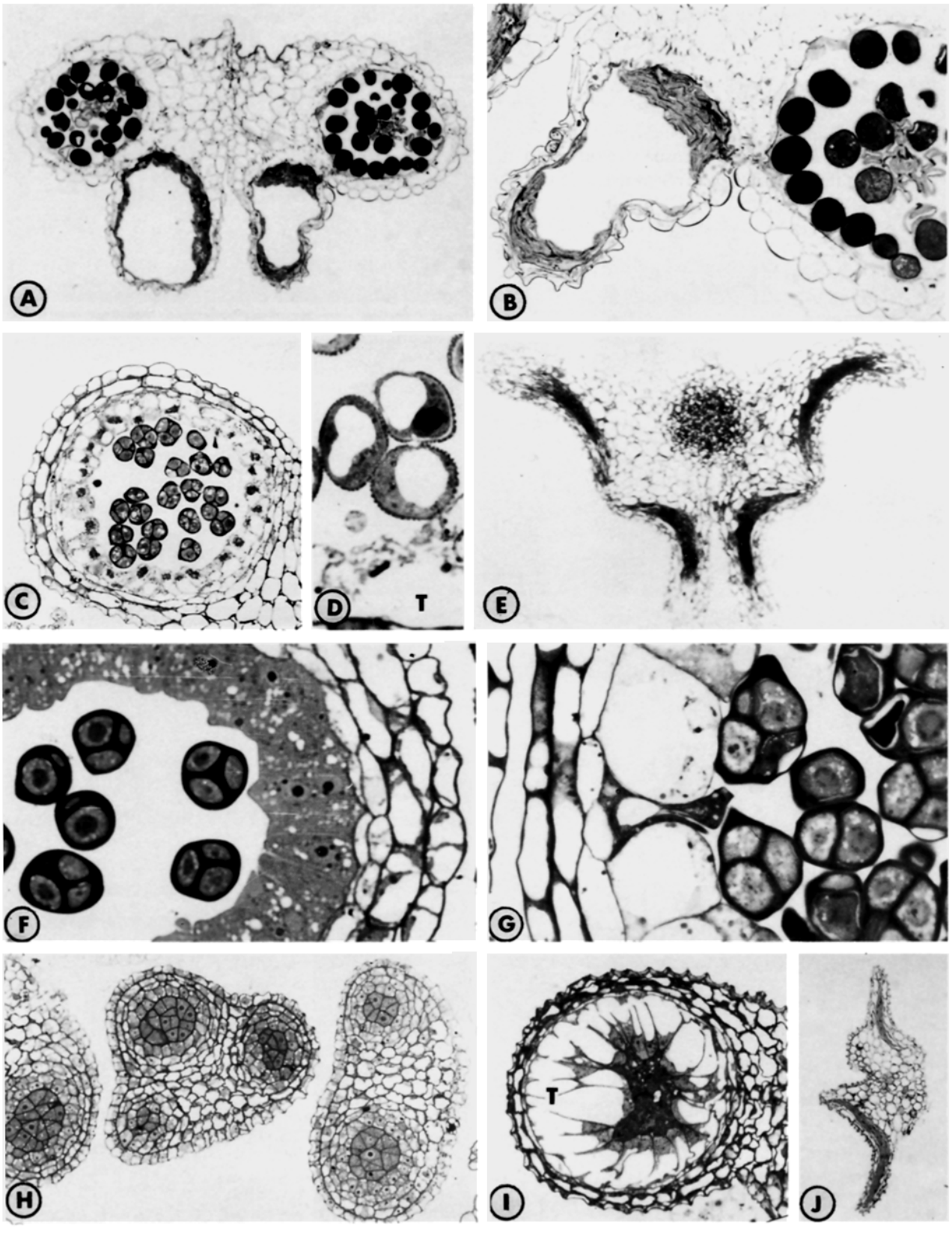
Cybrids 23-2, 23-3, and 23-5

Male sterility was variable in these three lines. In cybrid 23-2 the stamens often contained collapsed empty exines, but occasionally one locule showed a mixture of normal pollen grains and empty exines. This mixture was more generally observed in the stamens of cybrids 23-3 and 23-5. Sometimes the outermost microsporangia of the stamens produced essentially normal pollen grains, while the two innermost microsporangia of the same stamen produced only empty exines (Fig. 1 A, B). In this semi-sterile (or semi-fertile) stamen, a true endothecium only appeared close to the fertile microsporangia. The further the endothecial cell was situated from the normal pollen grains, the greater the decrease in size of the cell-wall thickenings; these thickenings were completely absent in the locule wall next to the ovary. Only the fertile locules opened.

Cybrids 27, 58 and 85

At the stage equivalent to anthesis in male-fertile flowers the six anthers appeared completely desiccated and shrunken (Fig. 2 E). Their filament was shorter than that found in fertile flowers at the same stage. When mounted in Alexander's stain, four longitudinal narrow green strips were seen in places corresponding to the four locules. These were easily extracted from the stamen, and when viewed by SEM (Fig. 2 H), they were observed to be comprised of empty, tricolpate, normally sculptured but flattened exines almost completely devoid of cyto-

Fig. 1 A–J. Light microscopy of 1-μm-thick, toluidine blue-stained sections. **A** Cybrid 23. Transverse section of semi-sterile anther. The two outermost microsporangia have formed pollen grains, while the two innermost only contain collapsed empty exines. ×90. **B** Cybrid 23. A larger view of the same section as depicted in **A** in which it is noticeable that cell wall thickenings are only present around the microsporangium with fertile contents. ×230. **C** Cybrid 58. Normal tetrads are present while the tapetum is abnormally light due to the presence of large vacuoles and clearing of the cytoplasm. ×250. Compare with the fertile line in **1F**. **D** Cybrid 58. The four sister microspores of the same tetrad remain close to each other. They have, however, reached a polarized, vacuolate stage. The tapetum (*T*) is almost completely autolysed. ×1,200. **E** Cybrid 58. Dessicated anther with four arcs of empty exines in the locules. ×220. **F** Normal line 'Bienvenu' at the tetrad stage showing the thick tapetum with binucleate cells. ×660. **G** Cybrid 77. Microsporangium at the tetrad stage. Note the excessive vacuolization of the tapetal cells and clearing of their cytoplasm. Compare with the fertile line in **1F**. ×880. **H** *Ogu* line. Transverse section of young flower bud showing anthers with a reduced number of microsporangia. ×220. **I** *Ogu* line. Microsporangium with excessive vacuolization of the tapetum (*T*) at late microspore stage. ×90. **J** *Ogu* line. Transverse section of a dessicated anther showing only two residual microsporangia. ×90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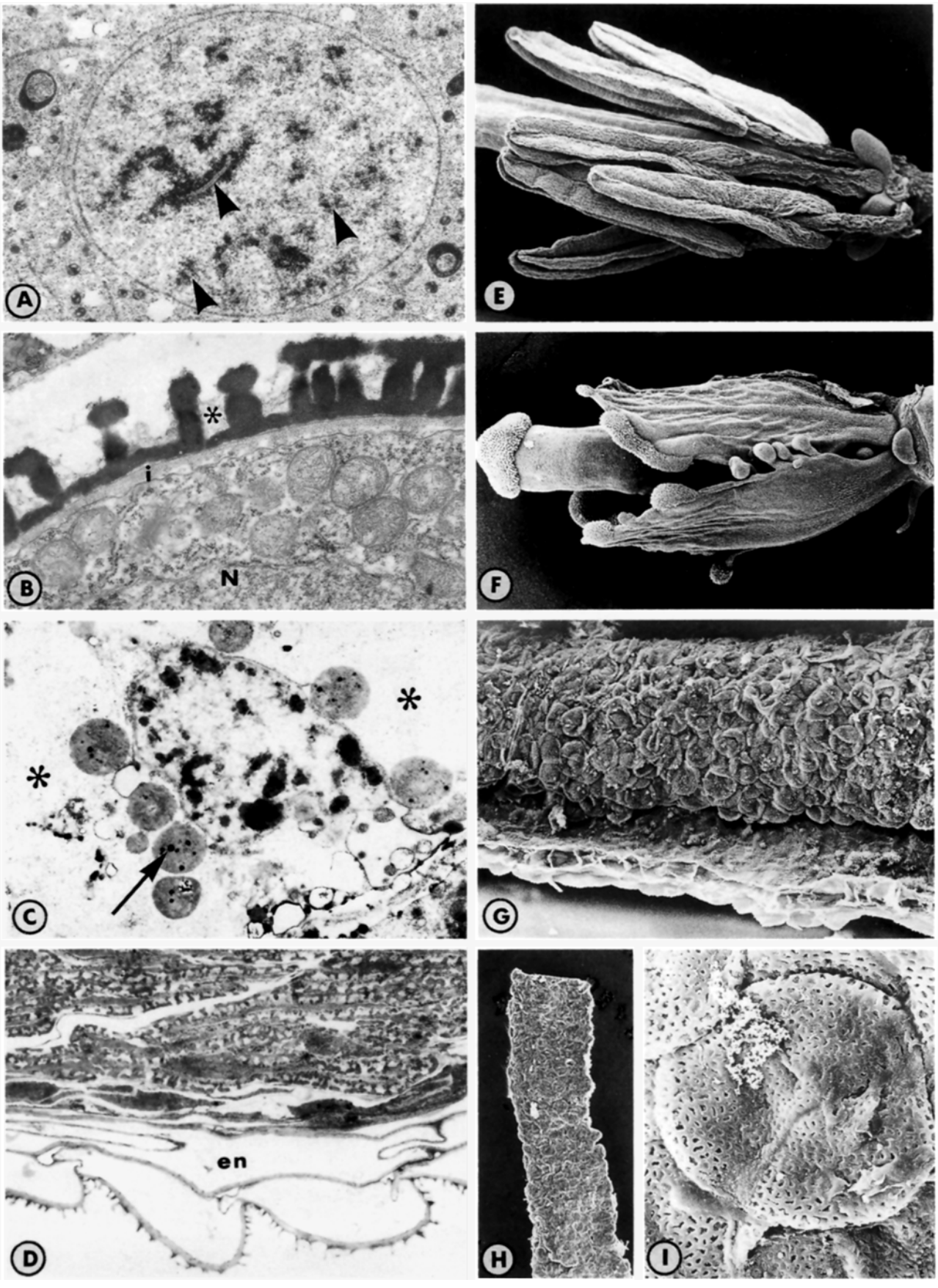
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plasm apart from very small residues (Fig. 2D, I). These green stips were also recognizable in semi-thin sections stained with toluidine blue (Fig. 1E). Their ektexine components, i.e. foot layer, columellas and tectum, appeared in TEM thin sections. The latter confirmed the absence of cell-wall thickenings in the subepidermal layer of cells. A true endothecium was never found in these indehiscent stamens.

The LM and TEM study of the developmental stages of these sterile stamens revealed the moment of death of the microspores and premature lysis of the tapetal cells. The appearance of sporogenous tissue was normal as was meiosis (fig. 2A) and tetrad formation inside the callose wall. Primexine and the usual tricolpate pattern were formed before the callose dissolved freeing the young microspores. Contrary to fertile anthers, the four microspores of the same tetrad did not always separate and often continued to adhere to each other. These microspores initially had a central nucleus, but they eventually became polarized and showed a laterally situated nucleus and the formation of the large vacuole (Fig. 1D). At this stage a progressive lysis of the microspore cell contents occurred. Only the sporopollenin material of the exine and a very thin fibrous layer corresponding to the intine remained. These ball-like empty sporoderms, subsequently flattened to give the green strips (Figs. 1E; 2G, H, I).

By the end of meiosis the binucleate cells of the tapetum showed one or two vacuoles that had enlarged considerably (Fig. 1C). On the outer side of these cells a normal electron-dense peritapetal membrane was formed, while the inner and radial cell walls cleared as in a fertile anther. But contrary to the latter, in the cybrid the tapetal cytoplasmic organelles disappeared prematurely with the cytosol becoming clearer and clearer (Figs. 1C, D; 2C). At the end of this process only a faint shadow of tapetal cells was left, and this adhered to the microspore cluster. The plastids of the tapetum never formed the large globules observed in fertile anthers (Fig. 2C). In the autolysing tapetal cells, during the tetrad stage, ribosomes were poorly represented. Mitochondria were often crowded together at the locular pole of the cell. During the last stage of tapetum lysis all of the membranes lost their continuity. Chromatin remnants of the nuclei persisted a short time, but eventually disappeared. This contrasted strongly with the behaviour of the microspores containing a normal cytoplasm and nucleus (Fig. 1C).

Cybrids 77 and 118

Teratological stamens with prominent feminization were observed, especially for cybrid 118 (Fig. 2F). These flower parts, often in reduced number (less than six), were flattened and vascularized like carpels or carpelloid leaves. They bore a stigma or stigmatoid regions at their tip and a variable number of ovules on their lateral lowest margins. The number and lengths of male sporangia in these bisexual flower parts were highly reduced. Their histology and development were identical to those previously described, including the premature death of tapetal cells, lysis of microsporal nucleus and cytoplasm at the vacuolate polarized stage, collapse of the exines and absence of cell-wall thickenings in the locule wall (Fig. 2D).

Ogu line

A reduced number and shorter lengths of microsporangia characterized this line (Fig. 1H, J). However, their abnormal developmental pattern at cellular level was of the same type as that described for cybrids 27, 58, 85, 77 and 118. It included the premature death of the tapetum, lysis of microspores followed by exine collapse and absence of a true endothecium (Fig. 1I).

Discussion

The results of our study on a number of cybrids with *ogu* cytoplasm confirm and define more explicitly the principle observations made by Ogura in 1968, e.g., an excessive vacuolization of the tapetal cells leading to their degeneration prior to the sudden collapse of the microspores. They are also in agreement with the light microscopy data obtained by Bartkowiak-Broda et al. (1979) using

Fig. 2A–D. TEM. **A** Cybrid 27. The nucleus of a meiocyte showing synaptonemal complexes (*arrows*). ×7,300. **B** Cybrid 27. Partial view of a polarized microspore, apparently normal and probably still alive, showing an exine with a fibrous material between the columellas (*asterisk*), an intine (*i*), a cytoplasm with numerous organelles and one nucleus (*N*). ×15,000. **C** Cybrid 58. Thin section adjacent to that viewed in **1C**. At the stage where tetrads of the microspores are present in the locules, there is a prominent vacuolization and degeneration of the tapetum. Plastoglobules remain small inside the plastids (*arrow*). No structured endoplasmic profile is present, and the cytosolic compartment between organelles is very clear (asterisks). ×9,250. **D** Cybrid 77. Thin section of collapsed empty exines in the dessicated anther at time of anthesis. Notice the absence of cell-wall thickenings in the endothecial layer (*en*). ×2,400. **E–I** SEM. **E** Cybrid 27. At a time corresponding to anthesis the six stamens are shrunken and completely indehiscent. Their filaments are shorter than those in fertile flowers. ×21. **F** Cybrid 118. In this flower the sepals and petals have been removed to show the feminization of the androecium. Stigmatoid regions are observed at the tip of these carpelloid flower parts, which bear ovules laterally near their base and which sometimes contain a reduced microsporangium. ×16. **G** Cybrid 27. An anther has been broken in order to view the clustered empty exines. ×360. **H** Cybrid 27. A strip of material can be removed from each locule. ×125. **I** Cybrid 27. Flattened empty exines adhering to each other. The apertural system and tectal surface of these exines are apparently normal when compared to fertile pollen grains. ×1,900

paraffin-embedded samples of rapeseed and corroborate many features noticed by Polowick and Sawhney (1991 a, b) (vacuolization of tapetum, complete abortion of microspores, carpelloidy and partial feminization). However, contrary to the latter, we did not observe a conspicuous reduction in sporopollenin deposits in the exines of microspores.

Ogu CMS is different from other CMS of *Brassicaceae*, such as the Bronowski system (Bartkowiak-Broda 1979), or the *ctr* CMS of oilseed rape studied by Grant et al. (1986). It also differs from the most commonly studied sterilities of main crops like corn (Lee 1976; Warmke and Lee 1977; Lee and Warmke 1979; Lee et al. 1979; Lee et al. 1980; Colhoun and Steer 1981), soybean (Albersten and Palmer 1979; Graybosch et al. 1984; Graybosch and Palmer 1987), sunflower (Horner 1977; Laveau et al. 1989), sorghum (Overman and Warmke 1972, Warmke and Overman 1972).

Our ultrastructural study points to three defects that occur along a time sequence at precise stages of development: first, the vacuolization and premature death of the tapetum beginning at the end of meiosis and soon completed (while fertile stamens retain lipidic tapetal materials until the trinucleate pollen stage); second, the sudden loss of cytoplasm and nucleus breakdown only when the microspores have reached their vacuolate stage; third, the indehiscence of the stamen due to the absence of a true endothecium with cell-wall thickenings and the persistence of the interlocule septum. This sequence was found in all the male-sterile cybrids included in this study.

Are these three events independent of each other and separately encoded in the genome or are they physiologically dependent? One could expect the early dysfunctioning of the tapetum and its subsequent complete desintegration to be sufficient to alter the subsequent development of the microspores, especially if we assume the trophic role attributed to this tissue (Echlin 1971; Vasil 1967; Heslop-Harrison 1968; Dickinson 1973; Mascarenhas 1975). However, it must be noticed that the microspores kept their cytoplasm and nucleus a long time after the tapetum had been lost. They seemed to grow normally in the locule, achieving their exine development and eventually becoming polarized as if they were normal, until this stage. Therefore, they can be considered to remain alive. This assumption is corroborated by the successful production of haploid plants from these microspores by anther culture at the late uninucleate stage of male-sterile lines of *ogu* or cybrid cytoplasm (unpublished data). In sterile stamens with *ogu* cytoplasm, the microspores received sufficient nutriments until their vacuolization. For further development of the microgametophyte a functional tapetum seems to be required *in vivo*.

Complete indehiscence of the stamens is a prominent feature of the archetypal expression of *ogu* CMS in radish stamens, and this was also found in the completely sterile locules of *Brassica napus* cybrids and *ogu* lines. Nevertheless, the unstable progeny of cybrid 23 provides material for studying the relationship between locule content and dehiscence. Since we found a true endothecium with U-shaped wall thickenings only around locules containing some apparently normal pollen grains, it appears that the microsporangium behaves as a functional unit where both centrifugal and centripetal movements of metabolites or growth regulators are to be expected. From a genetic viewpoint the instability of cybrid 23 progeny could mean that this cybrid may not possess the complete set of sterility genes of radish origin or that some of these genes could have their expression modified by physiological or environmental parameters.

A completely male-sterile pattern of abortion was found in cybrids 77 and 118. Both showed the three characteristic features of *ogu* CMS, i.e. premature decay of the tapetum, death of microspores at the vacuolate stage, indehiscence, and also a more or less pronounced feminization with reduction of the microsporangia. In the ontogeny of a stamen, early events must be impaired to produce these teratological, often bisexuate flower parts (Kaul 1989). Polowick and Sawhney (1987, 1991 a, b) have shown that a low temperature regime (18 °C–15 °C) or a moderate temperature regime (23 °C–18 °C) during bud development favors feminization of the androecium of *Brassica napus* with *ogu* cytoplasm. We have also observed feminization traits in some *B. napus ogu* lines in previous studies (unpublished data). Cybrids 77 and 118 also seem to possess these temperature-modifiable characters, while cybrids 23, 27, 58 and 85 probably do not since they never express a feminization of the androecium.

Cybrids 27, 58 and 85 showed the abortive pattern closest to the initial description of Ogura. Restoration experiments carried out on them (Pellan-Delourme 1986; Pelletier et al. 1987) indicated that they are more easily restored to fertility than cybrids 77 and 118. Thus, our light and electron microscopic study is in good agreement with the breeding experiments.

At the end of their paper concerning the same cybrids, Pelletier et al. (1987) assumed that "Ogura" mitochondrial DNA bears more than one factor and that cybrid recombinants possess fewer "factors". They also hypothesized that "Ogura" mitochondria lead to CMS because of two independent determinisms: the "Ogura" male sterility, already expressed in radish, together with an alloplasmic male sterility expressed when radish mitochondria are in the presence of a *Brassica* nucleus. Within the frame of this hypothesis we might be able to relate the abortion of microsporangia to the specific CMS expressed by *Raphanus sativus*, while size reduction and feminization of the androecium in *ogu* lines and in cybrids 77 and 118 might be more related to the alloplasmic situation. Another alloplasmic male sterility, with *Brassica napus* nucleus and *Diplotoxis muralis* cytoplasm, also shows an abortive pattern that includes feminization of the androecium (Pellan-Delourme and Renard 1987). The reduction in size of the androecium is determined at an early stage of the flower bud. Let us hypothesize that this step is the same as the one which differentiates towards a carpelloid morphology through a gene-controlled and hormone-mediated process. If we are right, it might be assumed that all of the cybrids have retained *ogu* CMS traits but that only cybrid 77 and 118 have kept feminization traits. This might be related to the mitochondrial DNA of these cybrids. Actually, cybrid 77 has radish mitochondria and cybrid 118 has mitochondria that are closer to those of radish than to those of rapeseed. On the contrary, the mitochondrial DNA of the other cybrids are closer to that of rapeseed (Chetrit et al. 1985; Vedel et al. 1986). Cybrid formation proceeds as a phenotype and genotype "sorter" (Fig. 3).

All of the studies on cybrids with respect to their various characteristics – nectar secretions (Mesquida

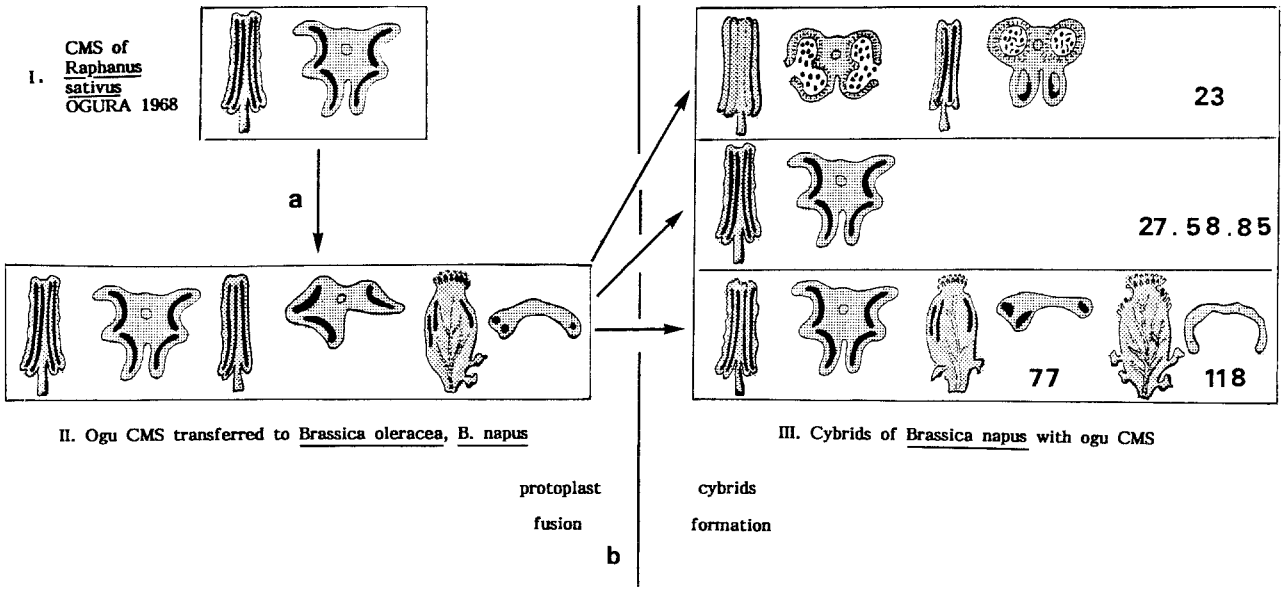

Fig. 3a, b. Expression of *ogu* CMS in *Raphanus* and *Brassica* (*ogu* lines and cybrids). **I** Radish archetype (Ogura description): the male-sterile flowers at anthesis had six shrunken stamens with four collapsed locules each; early breakdown of the tapetum followed by sudden degeneration of the microspores; complete indehiscence; quite stable and hereditary character. **a** Transfer to *Brassica oleracea* and then to *Brassica napus* (*ogu* lines). **II** Once transferred from *Raphanus* to *Brassica* an alloplasmic situation was created. The CMS now exhibited some variation in its expression, like feminization of the androecium or reduction in number and length of the microsporangia. **b** Protoplast fusion after plant regeneration led to different cybrids. **III** Our cytological and anatomical study showed cybrids 27, 58, 85 to have the same phenotype, one close to the radish archetype. Cybrid 23 was unstable, partially male fertile. Cybrids 77 and 118 have retained the feminization of the androecium due to alloplasmy. On the whole, cybrid formation proceeded as a phenotype and genotype sorter. Cybrids 27, 58 and 85 have eliminated some undesirable characters of alloplasmic origin

et al. 1991), patterns of abortion in the stamens, restriction patterns of mitochondrial DNA and genetics of male fertility restoration – are convergent. They define the same groups of cytoplasms. All of the results emphasize that protoplast fusion is a useful tool for recombining and sorting out cytoplasmic characters. It was thus possible to select cybrids that had eliminated the bad characteristics of the *ogu* lines (chlorophyll deficiency, low nectar secretions, feminization of the androecium), but kept the high stability of male sterility.

References

Albersten MC, Palmer RG (1979) A comparative light and electron microscope study of microsporogenesis in male sterile (M. S.) and male fertile soybeans (*Glycine max* L Merr). Am J Bot 66:253–265

Alexander MP (1969) Differential staining of aborted and non-aborted pollen. Stain Technol 44:117–122

Bannerot H, Boulidard L, Cauderon Y, Tempe J (1974) Cytoplasmic male sterility transfer from *Raphanus* to *Brassica*. Proc Eucarpia Crop Sect Cruciferae 25:52–54

Bartkowiak-Broda I, Rousselle P, Renard M (1979) Investigations of two kinds of cytoplasmic male sterility in rapeseed (*Brassica napus* L.) Genet Pol 20:487–4

Cerceau-Larrival MT, Duc G, Audran JC, Bouillot J (1982) La stérilité mâle nucléocytoplasmique chez la féverole (*Vicia faba* L.). IV. Incidence de la stérilité mâle nucleo-cytoplasmique 447 sur le phénotype du pollen à l'anthèse. Rev Cytol Biol Végét Bot 5:81–93

Chetrit P, Mathieu C, Vedel F, Pelletier G, Primard C (1985) Mitochondrial DNA polymorphism induced by protoplast fusion in Cruciferae. Theor Appl Genet 69:361–366

Colhoun CW, Steer MW (1981) Microsporogenesis and the mechanism of cytoplasmic male sterility. Ann Bot 48:417–424

Dickinson HG (1973) The role of plastids in the formation of pollen grain coatings. Cytobios 8:25–40

Echlin P (1971) The role of the tapetum during microsporogenesis of angiosperms. In: Heslop-Harrison J (ed) Pollen: development and physiology Butterworths, London, pp 41–61

Grant I, Beversdorf WD, Peterson RL (1986) A comparative light and electron microscopic study of microspore and tapetal development in male fertile and cytoplasmic male sterile oilseed rape (*Brassica napus*). Can J Bot 64:1055–1068

Graybosch RA, Palmer RG (1987) Analysis of a male-sterile character in soybeans. J Hered 78:66–70

Graybosch RA, Bernard RL, Cremens CR, Palmer RG (1984) Genetic and cytological studies of a male-sterile, female fertile soybean mutant. A new male sterile gene (ms2) in *Glycine max* (L.) Merr. J Hered 75:383–388

Kaul MLH (1988) Male sterility in higher plants. Monographs on theoretical and applied genetics vol 10. Springer, Berlin Heidelberg New York Tokyo

Heslop-Harrisson J (1968) Tapetal origin of pollen-coat substances in *Lilium*. New Phytol 67:779–786

Horner HT (1977) A comparative light-and electron-microscopic study of microsporogenesis in male-fertile and cytoplasmic male-sterile sunflower (*Helianthus annuus*). Am J Bot 64:745–759

Laveau JH, Schneider C, Berville A (1989) Microsporogenesis abortion in cytoplasmic male sterile plants from *H. petiolaris* or *H. petiolaris fallax* crossed by sunflower (*Helianthus annuus*). Ann Bot 64:137–148

Lee SLJ (1976) The cytology of pollen abortion in T (Texas) cytoplasmic male sterile corn (*Zea mays* L.) Ph. D. thesis, University of Florida

Lee SLJ, Warmke HE (1979) Organelle size and number in fertile and T-cms corn. Am J Bot 66:141–148

Lee SLJ, Gracen VE, Earle ED (1979) The cytology of pollen abortion in C-cytoplasmic male sterile corn anthers. Am J Bot 66:656–667

Lee SLJ, Earle ED, Gracen VE (1980) The cytology of pollen abortion in S cytoplasmic male-sterile corn anthers. Am J Bot 67:237–245

Mascarenhas JP (1975) The biochemistry of angiosperm pollen development. Bot Rev 41:259–314

Maurin N (1984) Anomalies cellulaires liées aux stérilités mâles de la féverole (*Vicia faba* L.) Mém DEA Sci Agron Univ Rennes 1984

Mesquida J, Pham-Délègue MH, Marilleau R, Le Métayer M, Renard M (1991) La secrétion nectarifére des fleurs de cybrides mâle-stériles de colza d'hiver (*Brassica napus* L). Agronomie 11:217–227

Ogura H (1968) Studies on the new male sterility in Japanese radish, with special reference to the utilization of this sterility towards the practical raising of hybrid seeds. Mem Fac Agric Kagoshima Univ 6:39–78

Overman MA, Warmke HE (1972) Cytoplasmic male sterility in sorghum. II. Tapetal behavior in fertile and sterile anthers. J Hered 63:227–234

Pellan-Delourme R (1986) Etude de deux systèmes de stérilité mâle génocytoplasmique introduits chez le colza (*Brassica napus* L.) par croisements intergénériques avec *Raphanus* et *Diplotaxis*. Thèse, Université de Rennes

Pellan-Delourme R, Renard M (1987). Identification of maintainer genes in *Brassica napus* L. for the male- sterility-inducing cytoplasm of *Diplotaxis muralis* L. Plant Breed 99:89–97

Pelletier G, Primard C, Vedel F, Chetrit P, Remy R, Rousselle P, Renard M (1983) Intergeneric cytoplasmic hybridization in Cruciferae by protoplast fusion. Mol Gen Genet 191:244–250

Pelletier G, Primard C, Vedel F, Chetrit P, Renard M, Pellan-Delourme R, Mesquida J (1987) Molecular, phenotypic and genetic characterization of mitochondrial recombinants in rapeseed. In: Proc 7th Int Rapeseed Conf. Poznan, pp 113–118

Polowick PL, Sawhney VK (1987) A scanning electron microscopic study on the influence of temperature on the expression of cytoplasmic male sterility in *Brassica napus*. Can J Bot 65:807–814

Polowick PL, Sawhney VK (1991a) Microsporogenesis in a normal line and in the ogu cytoplasmic male-sterile line of *Brassica napus*. I. The influence of high temperatures. Sex Plant Reprod 3:263–276

Polowick PL, Sawhney VK (1991b) Microsporogenesis in a normal line and in the *ogu* cytoplasmic male-sterile line of *Brassica napus*. II. The influence of intermediate and low temperatures. Sex Plant Reprod 4:22–27

Rousselle P (1982) Premiers résultats d'un programme d'introduction de l'androstérilité "Ogura" du radis chez le colza. Agronomie 2:859–864

Vasil IK (1967) Physiology and cytology of anther development. Biol Rev 42:327–373

Vedel F, Chetrit P, Mathieu C, Pelletier G, Primard C (1986) Several different mitochondrial DNA regions are involved in intergeneric recombination in *Brassica* cybrid plants. Curr Genet 11:17–24

Warmke HE, Lee S-LJ (1977) Mitochondrial degeneration in Texas cytoplasmic male-sterile corn anthers. J Hered 68:213–222

Warmke HE, Overman MA (1972) Cytoplasmic male sterility in sorghum. I. Callose behavior in fertile and sterile anthers. J Hered 63:105–106