Plant polytene chromosomes

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INTRODUCTION

Polytene chromosomes are structures found in highly specialized tissues in some animal and plant species, which are amplified through successive cycles of endoreduplication, finally producing several copies of each chromosome. For this reason, they have been very important in elucidating chromosome fine structure and physiology, especially in diptera.

In plants, polytene chromosomes have been observed in only a few species, and seemed to be restricted to ovary and immature seed tissues, e.g., in *Phaseolus coccineus* and *P. vulgaris* (Nagl, 1981), until relatively recently, when they were observed in the cells of the anther tapetum of *Vigna unguiculata* (Guerra and Carvalheira, 1994) and of some *Phaseolus* species (Carvalheira and Guerra, 1994). With the discovery of the polytenics in tapetum tissue, it was observed that in many other species of various angiosperm families the tapetal cells also display polytene, polyploid or both types of nuclei. In some species of *Phaseolus* and *Vigna* the polytenics are more clearly defined and, therefore, better suited to the study of this type of chromatin organization. It is, however, important to differentiate between the nuclear cycles that result in polyploid nuclei and those that produce polytene nuclei, because these two terms of the nuclear types are often used indiscriminately in the literature. In this paper some aspects of the occurrence of plant polytenes will be summarized along with the structure and function of these chromosomes.

ENDOMITOSIS AND ENDOREDUPLICATION

Nagl *et al.* (1985) described polytene chromosomes as giant chromosomes produced by changes in the mitotic cycle during the interphase stage. In such a modified nuclear cycle, the chromatin duplicates its DNA content during the G_1 and S stages, but, instead of passing to the G_2 stage, the nucleus initiates a new G_1 phase, thus starting a new cycle of chromatin duplication. This type of cycle was first described in 1939 by Geitler, as occurring in the somatic cells of the insect *Gerris lateralis* (Painter and Reindorp, 1939; D'Amato, 1964), and was named the "endomitotic cycle" because it develops within the nuclear envelop without either achromatic spindle formation or nuclear or cellular division (Nagl, 1970a; Brodsky and Uryvaeva, 1985). The term endomitosis is, however, generally used to describe the formation of both polyploid and polytene nuclei (q.v. Nagl, 1974). Nagl (1978, 1981, 1987) has suggested the term endocycle rather than endomitosis, and D'Amato (1984) has adopted the term endomitotic and endoreduplication to distinguish between those that produce polyploid and polytene nuclei, respectively.

The endomitotic cycle (endomitosis) starts with a normal prophase (endoprophase), after which the chromosome contracts further (endometaphase), their sister chromatids separate from each other (endoanaphase) and decondense to assume the interphase nuclear structure, resulting in polyploid cells, with double the chromosome number (endopolyploidy) at the end of each cycle. The essential difference between endomitosis and the normal cell cycle is the absence of nuclear membrane dissolution in endomitosis, with the whole cycle occurring inside the nucleus. Such cycles have been observed in the anther tapetum of some angiosperm species, as in some *Passiflora* species and in *Papaver rhoeas* (Figure 1a).

The endoreduplication cycle differs from endomitosis because it results in polytene cells (cells with many identical paired chromatids). In the endoreduplication cycle, the chromatid number is duplicated, but they do not segregate, and after various endoreduplication cycles, larger and thicker chromosomes are produced, called polytenics. In the endoreduplication cycle, the condensation and decondensation stages are not evident (D'Amato 1984, 1989), except in some cells where it is possible to see the chromocenter dispersion phase, known as the Z-phase (Nagl, 1970b, 1972; Cavallini *et al.*, 1981).

Depending on the behavior of the sister chromatids, polytene nuclei can be divided into two structural types. The first, and most well studied, are the chromosomes of the larval cells of *Drosophila*, Chironomidae and other diptera (Ashburner, 1970; Brodsky and Uryvaeva, 1985). These polytenics are characterized by numerous transverse bands along their linear axis, produced by the exact pairing of sister chromatids and the intimate association of their chromomeres (Ashburner, 1970). The somatic pairing of homologous chromosomes gives the false impression that there has been a decrease in chromosome number, because each nucleus appears to contain the haploid number of giant chromosomes.

The other structural type of polytene nuclei also has

the grouping of sister chromatid bundles resulting from several endoreduplication cycles, but in this case is characterized by the lack of any intimate pairing of the chromatids (Figure 1b). This nucleus type is observed more frequently, typical examples being found in the giant trophoblast cells of mammals (Nagl, 1985), the trophocit cells of many insects (Painter and Reindorp, 1939), some ovary tissues during the development of many angiosperms (Corsi *et al.*, 1973; Nagl, 1976) and in the anther tapetum of some plant species (D'Amato, 1984; Guerra and Carvalheira, 1994; Carvalheira and Guerra, 1994, 1998). In these nuclei, which can be recognized both by the large size of their chromocenters and by the diploid number of polytene chromosomes, the chromosome number does not appear to be reduced as in polytene-type nuclei. Another peculiar giant chromosome type, which likewise does not present somatic pairing, has been found in some ciliates (e.g., *Stylonychia mytilus*) that have a macronucleus with polytene chromosomes and a diploid micronucleus (Ammermann, 1971; Ammermann *et al.*, 1974). The polytenics of these ciliates display band and interband patterns (also seen in *Drosophila*), but the macronucleus disintegrates after its development while the micronucleus remains active.

It is interesting to note that the endocycles are not processes of cell multiplication but are associated with cell differentiation and seem to be genetically controlled, with
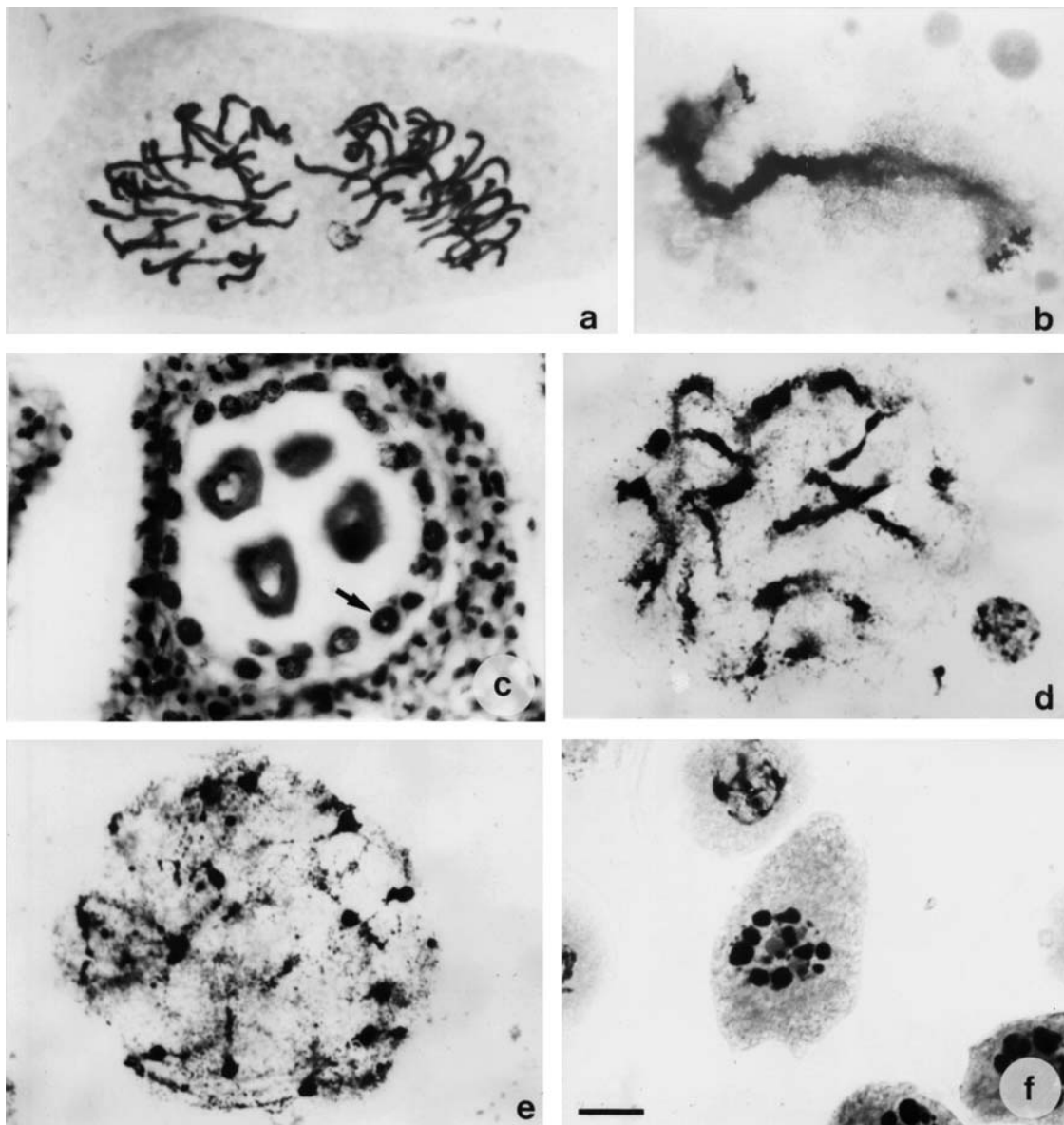

Figure 1 - a) Binucleate tapetal cell of *Papaver rhoeas*; **b**) C-banding in polytene chromosome of *Phaseolus coccineus* suspensor (Courtesy of Dr. D. Schweizer); **c**) transversal section of *Vigna unguiculata* anther with meiocytes (arrow shows tapetal nucleus); **d**) tapetal nucleus of *Vigna unguiculata* showing polytene chromosomes (compare with diploid nucleus); **e**) tapetal polytene nucleus of *Phaseolus acutifolius* showing chromocenters-associated thinner chromatin bundles; **f**) mononucleate tapetal cell of *Genipa americana* showing chromocenters without chromatids bundled. Bar scale represents 10 μm, except to **b** and **c**.

both endopolyploidy and polyteny leading to cell specialization in certain tissues. These nuclei have generally been observed in ephemeral tissues made up of only a few cells with intense metabolic activity, the main function of which is to provide nutritional support to vital organs during certain periods of development (e.g., the larval salivary glands of insects, the mammalian trophoblasts and the embryo suspensor cells of angiosperms). In such tissues, the cytoplasmatic volume and nuclei DNA content of the cells are increased by endomitosis or endoreduplication cycles (Nagl, 1974, 1985; Nagl *et al*., 1985).

OCCURRENCE OF POLYTENE CHROMOSOMES

Polytene nuclei were first observed in the larval salivary glands of Chironomidae, by Balbiani in 1881, but only at the beginning of the 1930s did Heitz and Bauer & Painter, independently and simultaneously, rediscover these enormous nuclei in the Malpighian tubules of *Bibio hortulanus* and in the larval salivary glands of *Drosophila melanogaster*, respectively (Ashburner, 1970). A few years later, Koltzoff, in 1934, and Bauer, in 1935, proposed the term polytenics for the giant chromosomes observed in these nuclei (Ashburner, 1970); polytene cells have since been described in many species (Nagl, 1978; Brodsky and Uryvaeva, 1985; Carvalheira and Guerra, 1998).

In plants, the first giant nuclei were observed by Osterwalder in 1898, in the enormous antipodal cells (antipodes) of the embryo sac of *Aconitum* (Nagl, 1981). However, as with the discovery of the giant cells of Chironomidae, the antipodal nuclei were largely forgotten for about 60 years. Only in 1956 did Tschermack-Woess and collaborators, during a reappraisal of the genus *Aconitum* genus and other plant species, recognize that the chromosomes observed in the antipodes were polytenics (Nagl, 1981). Unlike *Drosophila* polytene chromosomes, which present numerous bands and interbands, plant polytenics have a granular and fibril structure with no distinct bands (see Figure 1). This structure probably occurs because of the absence of intimate synapsis between the sister chromatids. It is also believed that the chromocenter dispersion phase (Z phase) has some influence on the morphology of plant polytenics, as it results in a slight separation of these chromatids (Nagl, 1970a). However, Nagl (1969a) has reported that in *Phaseolus vulgaris* the structure of polytene chromosomes of embryo suspensor cells seems to be altered when these cells are submitted to low temperatures, becoming partly compacted and forming bands similar to those seen in *Drosophila*. Such results have not been observed again, remaining the only report of plant polytenics with bands and interbands.

Since the discovery of the polytene nuclei in antipodes, many other tissues composed of polytene cells have also been described (Table I). It is interesting to note that, until very recently, the cells with polytene chromosomes seemed to be limited to ovary tissues (antipodal cells, synergids, endosperm and embryo suspensor cells); however, polytenics have now been observed in anther hair, glandular hair and anther tapetal cells (Table I).

Polyteny can also be induced *in vitro* and it has been found that the meristematic tissues of root tips and the cotyledon cells of some plant species are able to form polytene chromosomes when submitted to specific treatments, including high temperatures (Shang and Wang, 1991) or an appropriate amount of certain growth regulators (mainly auxin and cytocinin) in the medium (Marks and Davies, 1979; Therman and Murashige, 1984).

POLYTENE CHROMOSOMES OF EMBRYO SUSPENSOR

The most widely studied plant tissues with polytene cells is the *Phaseolus* embryo suspensor tissue. This tissue is found in the developing ovary of several angiosperm species (Esau, 1974). In *P. coccineus* and *P. vulgaris*, the suspensor is composed of about 200 cells, distributed between the basal and junction regions. The basal region is formed of about 20 giant mononucleate cells with a high level of polytenization, and with the DNA content of some cells being up to 8.192 C (Brady, 1973a,b). The junction region is composed of about 180 cells linking the basal region to the embryo proper. This last region possesses polyploid cells and/or cells with low polyteny level (Brady and Clutter, 1974).

The embryo suspensor provides nutritional support for the immature embryo, supplying proteins or synthesizing the substances necessary for embryo development (Schulz and Jensen, 1969). The underdevelopment of the cell wall of suspensor cells and their other structural characteristics indicate their secretory function in transporting nutrients through their membranes to the embryo (Nagl, 1974; Cionini, 1987). Analyses of the growth regulator level and transcription activity indicate that the suspensor tissue may play an important role during embryo ontogenesis, and seems to have a function in the synthesis of phytohormones needed for embryo development (Walbot *et al*., 1972; Clutter *et al*., 1974; Alpi *et al*., 1975; Cionini *et al*., 1976; Lorenzi *et al*., 1978).

The basal cells of the embryo suspensor tissue of *P. coccineus* display 22 polytene chromosomes that are up to 30 times larger than the mitotic ones (Nagl, 1974), the 11 chromosome pairs having been identified earlier by their heteropycnosis pattern (Nagl, 1967). All mitotic chromosomes present heterochromatic centromeric bands and some weak interstitial and terminal ones (Schweizer and Ambros, 1979). Staining with the fluorochromes CMA and DAPI has revealed that most of these bands are CMA[+], although unlike mitotic bands they contain a small amount of DAPI[+] heterochromatin (Schweizer, 1976). The difference observed in the fluorescent pattern has been attributed to the better structural resolution of the giant chromosomes.

Table I - Occurrence of polytene chromosomes in plants.

Tissue	Species	References
Antipodes	*Aconitum neomontanum*	Nagl, 1981
	Clivia miniata	Nagl, 1981
	Scilla bifolia	Nagl, 1976
Endosperm	*Phlomis viscosa*	Enzenberg, 1961
	Rhinanthus alectrophus	Nagl, 1981
	Thesium alpinum	Nagl, 1981
Anther hair	*Bryonia dioica*	Nagl, 1981
Glandular hair	*Salvia horminum*	Gostev and Asker, 1978
Synergids	*Allium ursinum*	Nagl, 1978
Embryo suspensor	*Brassica nigra*	Viegi *et al.*, 1976
	Eruca sativa	Corsi, 1972; Corsi *et al.*, 1973
	Gagea lutea	Nagl, 1981
	Lotus carmeli	Freed and Grant, 1976
	L. pedunculatus	Freed and Grant, 1976
	L. pursbianus	Freed and Grant, 1976
	Phaseolus cafter	Nagl, 1981
	P. coccineus	Clutter and Sussex, 1969
	P. hysterinus	Nagl, 1974
	P. multiflorus	Nagl, 1974
	P. tuberosus	Nagl, 1974
	P. vulgaris	Nagl, 1969b,c
	Psophocarpus tetragonolobus	Sen, 1980
	Tropaeolum majus	Nagl, 1981
Anther tapetum	*Arachis hypogeae*	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Caesalpinia echinata	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Clitoria cajanifolia	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Crotalaria retusa	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Genipa americana	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Habenaria hexaptera	Carvalheira, G.M.G. and Guerra, M., unpublished data
	H. obtusa	Carvalheira, G.M.G. and Guerra, M., unpublished data
	H. repens	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Indigofora hirsuta	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Lathyrus sp.	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Lupinus polyphyllus	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Luffa cylindrica	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Macroptilium peduncularis	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Phaseolus acutifolius	Carvalheira and Guerra, 1994
	Phaseolus coccienus	Carvalheira and Guerra, 1994
	Phaseolus lunatus	Carvalheira and Guerra, 1994
	Phaseolus microcarpus	Carvalheira and Guerra, 1994
	Pithecellobium dulce	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Pisum sativum	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Sesbania marginata	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Sophora tomentosa	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Tropaeolum majus	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Vicia sp.	Carvalheira, G.M.G. and Guerra, M., unpublished data
	Vigna radiata	Carvalheira and Guerra, 1998
	Vigna umbellata	Carvalheira and Guerra, 1998
	Vigna unguiculata	Guerra and Carvalheira, 1994
	Vigna vexillata	Carvalheira and Guerra, 1998
	Zomicarpa riedeliana	Carvalheira, G.M.G. and Guerra, M., unpublished data

In situ hybridization experiments, with isotopic (q.v. Schumann *et al.*, 1990) and non-isotopic markers (q.v. Nenno *et al.*, 1994), have contributed considerably to the characterization of polytenics. These techniques have permitted both the location of many of their DNA sequences and the study of their replication cycle (Brady and Clutter, 1974). For example, the cytolocalization of the ribosomal genes in *P. coccineus* has been demonstrated by the use of RNAr-H[3], revealing RNA puff activity both in satellite pairs and in the heterochromatin of chromosome pairs, without satellites

(Avanzi *et al.*, 1971, 1972; Durante *et al.*, 1977). Isotopic techniques have also made it possible to observe the "extra nucleoli" associated with the telomeres of the polytenics without satellites, suggesting the existence of amplification in this region (Nagl, 1973). Actually, the extra DNA synthesis in the polytenics of the *Phaseolus* suspensor occurs at the beginning of embryogenesis and not simultaneously with the endoreduplication cycles (Avanzi *et al.*, 1970). Such gene amplification can occur both in the ribosomal cistrons and other regions of the genome and involves some polytene

chromosome chromatids (Cremonini and Cionini, 1977). Some of these amplified regions are released from the polytenics to form micronucleoli. According to Avanzi *et al.* (1971), the micronucleoli are composed of a spherical mass of ribonucleoprotein covered by a layer of DNA. These micronucleoli seem to be associated with the intense metabolism of the suspensor basal cells (Nagl, 1973).

With advances in the fluorescence *in situ* hybridization technique (FISH), several other sequences have been located in plant polytene chromosomes. The first genic sequence hybridized in polytenics was that of the *phaseolin* group (Schumann *et al.*, 1990; Nenno *et al.*, 1993, 1994). This gene encodes the main seed storage protein of *Phaseolus* species. In these papers, it was demonstrated that the *phaseolin* gene seems to be located in chromosome 7 of *P. coccineus*. Another gene that has been located in the polytenics of *P. vulgaris* is the *PGIP* gene which encodes for polygalacturonase-inhibiting protein, a cell wall protein that specifically inhibits fungal endopolygalacturonases that are important during the early stages of plant pathogenesis. The *PGIP* gene has been located in a single region of the pericentromeric heterochromatin of the chromosome pair X, next to the euchromatin (Frediani *et al.*, 1993).

The FISH technique as applied in polytene chromosomes has also been a useful tool to study gene evolution. Nagl (1991) hybridized telomeric DNA and the *aromatase* gene sequence (both from human genome) in *P. coccineus* and *P. vulgaris* polytenics. The results showed that these sequences also hybridize with plant chromosomes, supporting the hypothesis of the evolutionary conservation of important coding or non-encoding sequences throughout living organisms.

POLYTENE CHROMOSOMES OF ANTHER TAPETUM

Polytene chromosomes are also observed in other plant tissues, of which the anther tapetum tissue has made valuable contributions to the understanding of polytenics in angiosperms. This tissue is widely conserved, being found in groups ranging from bryophytes to angiosperms (Pacini and Franchi, 1993).

The tapetum is the innermost layer of the anther wall in close contact with the pollen grains (Figure 1c). It is generally composed of a simple layer of cells, characterized by the presence of dense cytoplasm and quite a well-developed nucleus (Echlin, 1971). During the differentiation of the tapetum, the cells increase both their cytoplasmatic and nuclear volume and then undergo autolysis and degenerate (Mascarenhas, 1990). The tapetum's function seems to be related to the maturation of pollen grain, with biochemical and cytological studies demonstrating the intense metabolic activity in its cells at the end of the tetrad stage and during exine formation (Rowley, 1993).

Until recently, most of the information on the nuclear development of the tapetal cells has come from studies of anther ontogenesis, based on histological analyses by optical or electronic microscopy. Cytological analyses in tapetum were done mainly by Cooper (1933), Brown (1949), Oksala and Therman (1977), Franceschi and Horner (1979), and D'Amato (1984, 1989). However, cytogenetical analysis of the tapetal cells of *Vigna* species has revealed that this tissue can present very peculiar characteristics (Guerra and Carvalheira, 1994). Anther tapetum cells are characterized by the presence of endomitotic or endoreduplication cycles (Cooper, 1933; D'Amato, 1984, 1989; Malallah *et al*, 1996). In species where the tapetum layer is composed of mononucleate cells, the increase in DNA content is generally a consequence of several endoreduplication cycles, while in species with bi- or multinucleate cells in the tapetum it is the endomitotic cycle which is responsible. In spite of the endoreduplication cycle producing mononucleate cells, tapetal binucleate cells with polytene nuclei have sometimes been observed (Carvalheira and Guerra, 1994).

In general, at the beginning of meiosis, the tapetal cells are mononucleate and diploid. During the tapetal differentiation, three cellular types can be observed, i.e. multinucleate cells, with more than one diploid nucleus (Malallah *et al.*, 1996), mononucleate cells, with a single polyploid nuclei (Carvalheira, G.M.G. and Guerra, M., unpublished data), and mono- or binucleate cells, with one or two polytene nuclei (Carvalheira and Guerra, 1994). In each of these cases, the DNA content per cell is often increased, suggesting that this tissue needs several copies of most genes to supply specific substances for exine development and consequent pollen grain maturation.

The increase in the ploidy level is probably caused either by suppression of anaphase movement (producing a "dumbbell"-shaped polyploid interphase nucleus) or by the occurrence of endomitotic cycles (D'Amato, 1989), while the increase in the number of nuclei per cell is due to the occurrence of one or more mitosis cycles without cytokinesis, resulting in multinucleate cells. Polytene nuclei, on the other hand, are formed through the endoreduplication cycles, and could remain in the interphase stage until the S phase, or progress to the prophase stage and return to a new G_1 phase (Guerra and Carvalheira, 1994).

Analysis of both ploidy level and nuclear structures in tapetal cells in genera of several subfamilies has revealed that their chromatin structure may be constant at the genus level. In the family Scrophulariaceae, for example, some species of the genera *Pedicularis* and *Melampyrum* have tapetal cells with tetraploid nuclei, in the post-meiotic period. On the other hand, in this same family, the genera *Odontetis*, *Euphrasia* and *Bellardia* have nuclei with enormous chromocenters, but with the same ploidy level (Greilhuber, 1974).

Like the other plant tissue with polytene chromosomes, anther tapetum cells can display polytenics that vary in structure and morphology from species to species (q.v.

Nagl, 1974, 1981; Carvalheira and Guerra, 1994, 1998). For example, polytene nuclei in the antipodes of *Papaver rhoeas* were classified by Nagl (1981) into four different types, i.e., nuclei with chromocenters associated with radial chromatin bundles; decondensed nuclei with isolated chromatin fibers; nuclei with condensed chromatin, and nuclei with polytenics proper. Similar variation was found in suspensor cells of *Phaseolus* embryos, where the polytenics sometimes had granular or fibril form, depending on the degree of contraction in the interchromomeres (Nagl, 1978).

According to Carvalheira and Guerra (1998), the chromatin structure of the polytene nuclei in tapetal cells may basically be divided into three different types:

1. Individualized polytene chromosomes whose chromatin bundles are heteropycnotic in the proximal region and dispersed in the distal region (Figure 1d), characteristic of *Phaseolus coccineus, P. vulgaris* (Carvalheira and Guerra, 1994), *Vigna unguiculata* (Guerra and Carvalheira, 1994; Carvalheira and Guerra; 1998), *V. umbellata, V. radiata* (Carvalheira and Guerra, 1998), *Lathyrus* and *Sesbania marginata*.

2. Polytene nuclei with chromocenters associated with the chromatin bundles (Figure 1e), found in most of the species analyzed, including *Arachis hypogeae, Caesalpinea echinata, Clitoria cajanifolia, Crotalaria retusa,* in three *Habenaria* species, *Luffa cylindrica, Macroptilium peduncularis,* two *Phaseolus* species (Carvalheira and Guerra, 1994), *Pithecellobium dulce, Pisum sativum, Sophora tomentosa, Tropaeolum majus* and *Zomicarpa riedeliana*.

3. Polytene nuclei with chromocenters unassociated with chromatin bundles (Figure 1f), a less frequent type, characteristic of *Genipa americana, Indigofera hirsuta, Lupinus polyphyllus, Vigna vexillata* (Carvalheira and Guerra, 1998) and *Vicia* sp.

Of these three types of chromatin organization, only the first is ideal for karyological analyses. The polytenics of this group is generally individualized and condensed. This type of chromatin organization has allowed good chromosome spreads to be obtained, facilitating the chromosome counting. In most *Phaseolus* and *Vigna* species analyzed that had this type of organization, it was possible to observe all 22 chromosomes of the karyotype (q.v. Guerra and Carvalheira, 1994; Carvalheira and Guerra, 1994).

Although this type of chromatin organization seems to be ideal for karyological analyses, these polytene chromosomes are somewhat smaller than those observed in the embryo suspensor of *Phaseolus* or those of some other genera (compare Figure 1b and d). The largest polytenics of the anther tapetal cells (observed in *Vigna unguiculata*) are about 3.5 times bigger than the mitotic ones (Guerra and Carvalheira, 1994). The difference in size observed between polytenics of the embryo suspensor and anther tapetum is probably related to the number of endoreduplicated cells present in each of these tissues. As was mentioned previously, only a few basal cells undergo many endoreduplication cycles in the embryo suspensor tissue (Nagl, 1981), while in the anther tapetum hundreds of cells undergo this process. The low level of chromatin endoreduplication associated with a large number of cells seems to satisfy the metabolic necessities of both the anther tapetum and microspores. On the other hand, in the giant cell of the embryo suspensor, many endoreduplication cycles seem to be necessary to maintain the perfect functional and nutritional stage of the embryo, most of whose nutrients are supplied by the suspensor cell.

Although the polytenics of the anther tapetum are reduced in size, they have helped in the cytolocalization of DNA sequences. The first *in situ* hybridization in tapetal polytenics has revealed interesting data different from that which was observed in the polytene chromosomes of the embryo suspensor (Guerra and Kenton, 1996). As stated earlier, after *in situ* hybridization with the human telomere DNA probe, Nagl (1991) observed that the embryo suspensor polytenics of *Phaseolus* show a group of dots or compact bands at the telomeres. However, when synthetic telomere oligomers were hybridized with tapetum polytenics in an amphidiploid hybrid of *Phaseolus*, the oligo was preferentially located at, or close to, the chromocenters. These fluorescent areas were distributed randomly in the nuclear area, although association with the nuclear boundary was never observed (Guerra and Kenton, 1996). This may suggest that, at least in some aspects, the basic molecular organization of diploid nuclei in the anther tapetum is not completely conserved after the endoreduplication cycles. In fact, the loss of telomere association with the nuclear membrane has been documented in some special chromosome types, such as pigeon lampbrush chromosomes (Solovei and Macgregor, 1995) and Diptera polytenic chromosomes (Agar and Sedat, 1983). In plant polytene nuclei, however, this loss of telomeric association with the nuclear envelope was reported for the first time in anther tapetum polytenics (Guerra and Kenton, 1996).

These chromosomes have also helped in the identification of the 45S ribosomal sites in *Phaseolus coccineus* and *Vigna unguiculata* (Guerra *et al.*, 1996), and 5S sites, in *V. radiata* and *V. unguiculata* (Carvalheira *et al.*, 1998). In *P. coccineus*, six ribosomal sites were observed in tapetal cells, as has been reported previously (see Avanzi *et al.*, 1972; Durante *et al.*, 1977). Surprisingly, however, ten ribosomal sites were observed in *V. unguiculata* tapetum polytenics (Guerra *et al.*, 1996), instead of one or two pairs as earlier reported (Frahm-Leliveld, 1965; Barone and Saccardo, 1990; Galasso *et al.*, 1992). The large number of ribosomal sites observed in *V. unguiculata* when compared with *Phaseolus* has suggested that this increase in ribosomal sites may have been initiated by genetic mechanisms, such as gene conversion. According to Guerra *et al.* (1996), the variation in the number of rDNA sites observed between species of related taxa could be due to the differ-

ential amplification and fixation of rDNA sequences at different chromosomal sites. On the other hand, four 5S ribosomal sites were observed in *V. radiata* and *V. unguiculata* (Carvalheira *et al.*, 1998), confirming the previous reports for *V. unguiculata* (Galasso *et al.*, 1995), although these reports were first published for *V. radiata*.

In conclusion, although the polytenics of the anther tapetum are smaller than those in suspensor cells, both the large number of polytene cells in this tissue and their structural polytene morphology make these chromosomes more convenient for the study of plant polyteny and chromosome organization. Guerra and Carvalheira (1994) and Carvalheira and Guerra (1994, 1998) have suggested that such chromosomes present cycles of diffuse and condensed stages. The change from diffuse to condensed stage seems to depend on the endoreduplication level, genetic background and environmental factors. All these observations suggest that bundled polytene chromosomes of plants, at least in tapetal cells, are most probably the consequence of advanced endoreduplication cycles resulting in prophase or prophase-like chromosomes that may still be able to perform some DNA and RNA synthesis (Brady and Clutter, 1974; Cionini *et al*, 1982).

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