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Unusual cytological patterns in microsporogenesis in a cultivar of Fuchsia

1. Multiple spindle

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Summary. Multiple and multipolar spindles are a generalized feature of microsporogenesis in a cultivar of Fuchsia. Only the first meiotic division occurs and gives rise to sporads with nine microspores. Variation in chromosomal complements of the microspores is illustrated by pollen polymorphism. Since some of these pollen grains are able to germinate, the possible breeding value of this super-reductional type of division is questionable. Hypotheses concerning this phenomenon found in the literature are discussed in the light of our results.

Key words: Fuchsia microsporogenesis – Multiple spindle – Chromosomal instability

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Introduction

Some meiotic irregularities can be affected by chromosome homologies, genetic factors, cellular and external environment. These meiotic behaviors are well known.

For meiotic disturbances related to the spindle, the data are as yet scattered and fragmentary. The significance of these phenomena remains obscure. However, chromosomal instability is becoming a topic of increasing interest since somatic cell hybridization leads to symmetric and asymmetric hybrids (Hoffman and Adachi 1981). Chromosomal instability originates in both somatic or meiotic divisions as a result of the same causal factor: spindle disorganization.

A selective elimination of one genome set with respect to the other has been observed in *Hordeum* interspecific hybrids. This elimination of chromosomes was specific and gradual during the early development of the embryo (Bennett et al. 1976).

For the spindle-linked aberrations found in meiotic divisions, the nature of these disturbances is, according to the literature, of two kinds: multipolar spindle or multiple spindle. Multipolar spindle is the more frequent abnormality. Clark's study (1940) in *Zea mays* illustrates the changes that can be induced in the structure of the spindle. Arising from the result of u.v. irradiations the gene divergent (dv), when homozygous, causes the spindle in pollen mother cells to take an abnormal form. The usual picture obtained is that of a spindle that diverges and flares at the poles rather than one that converges to form a biacuminate structure. In some cases the spindle elongates greatly, and in doing so, follows the contours of the cell. The result is that several nuclei rather than two are formed in telophase. The second division which follows is normal or abnormal, since each nucleus, regardless of the number of chromosomes it may contain, forms its own spindle, which may or may not be divergent in character. More than four spores are sometimes formed from each microsporocyte, but when only four result, many of these are multinucleate. The fact that a study of seven plants showed that the range was from 13 to 90% suggests that the expression of the dv gene is altered by various environmental factors, or that the genetical background in which it is found determines its degree of effectiveness in altering spindle shape.

Divergent spindles also occur in multiploid sporocytes after cytomixis in Barley (Smith 1962).

Tripartite separation of the chromosome complement, which results in three unequal instead of two equal groups of chromosome at anaphase I, has been interpreted as "incomplete splitting of the spindle" (Vaarama 1953; Gottschalk 1958). Multipolar meiotic division has been detected in *Agropyron cristatum* (Tai 1970) and *Mimulus glabratus* (Tai and Vickery 1972) in *Carthamus tinctorius* (Carapetian and Rupert 1977).

The first ones to report multiple spindles were Beadle and McClintock (1928), also in *Zea mays.* Studying a collection of maize carrying factors of male sterility, they describe the microsporogenesis as: "Irregularities in the appearance of metaphase I increases with an increase in number of univalents. A microsporocyte with ten bivalents in metaphase I appears normal. When univalents are observed they do not always lie in one spindle. Usually there is one major spindle containing the several bivalents, when present, plus some of the univalents and one to several spindles containing one or more univalents. In consequence of the presence of several spindles, the sporocyte is divided into a number of unequal cells after the first meiotic division. Each cell contains one or more nuclei and each nucleus contains one or more chromosomes. These cells undergo a second meiotic division to form microspores. It is obvious that most of these microspores and the pollen grains formed from them do not contain a normal haploid set of chromosomes, and they are probably non-functional."

Multiple spindles have also been reported by Thompson (1962), in some higher polyploids of *Rubus*, and by Vasek (1962), in *Clarkia exilis*.

These spindles' irregularities are sporadic: some in percentages of sporocytes, 3.1% (Swanson and Nelson 1942), 5–10% (Tai and Vickery 1972), or portions of some anthers in a few buds of each variety (Thompson 1962). Working on a breeding program of horticultural fuchsias (Tilquin and de Brouwer 1982, Tilquin et al., in press), our attention was attracted to pollen polymorphism and to more than four spores at the sporad stage.

Materials and methods

Fuchsia cv 'Riccartonia' belongs to a collection living under controlled conditions in a greenhouse (70–80% humidity and 15–25 °C).

Controls has been made regularly to assure that meiotic process is normal in wild fuchsias.

The buds were fixed in acetic-acid-ethylalcohol (1:3) and stored in ethylalcohol (70%) at 4 °C.

The acetocarmin-squash method was employed but the staining of the chromosomes was very bad and so, phase contrast was used for the examination and documentation.

Results

All anthers excised on different days to demonstrate the constancy of the phenomenon show a uniform pattern of microsporogenesis.

"Meiosis cycle" is very short and so, within the anthers of one bud we can find all the stages, from MI to the sporad stage.

Figure 1 consists of three photographs of the same sporocyte at different focusing planes. The sporocytes are gently squash in order to avoid artefacts. Multiple spindle and multipolar spindles are obvious (Diagram 1).

A large variation in chromosome numbers per spindle exists: several microspindles with one bivalent, one more important with three bivalents, and multipolar (tetra- and hexapolar) spindles involving numerous bivalents and some multivalents. Chromosome number was estimated to 2n = c.140. The presence of spindles is suggested by the shape and the orientation of the bivalents and confirmed by electron microscopical studies. Furthermore, at this level, convergent microtubules have been seen.

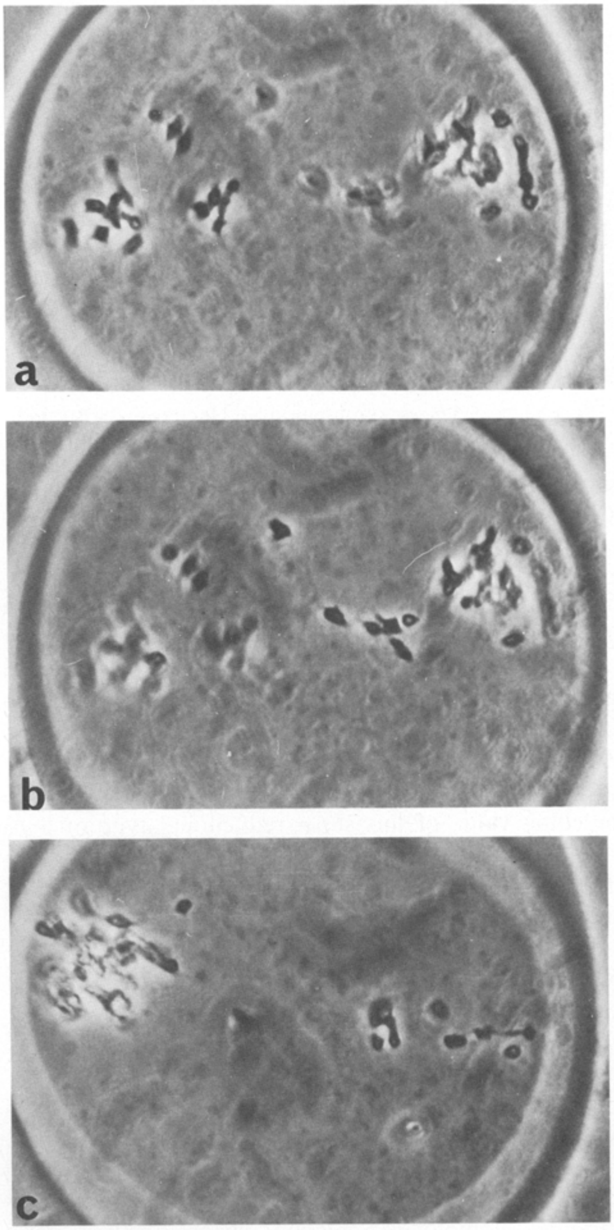

Fig. 1a–c. Metaphase of the same sporocyte at three focusing plane-phase contrasts (× 1,260)

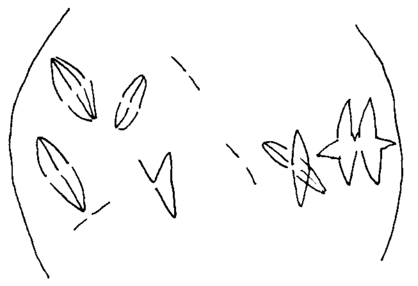

Diagram 1. Explanation of Figs. 1a–c

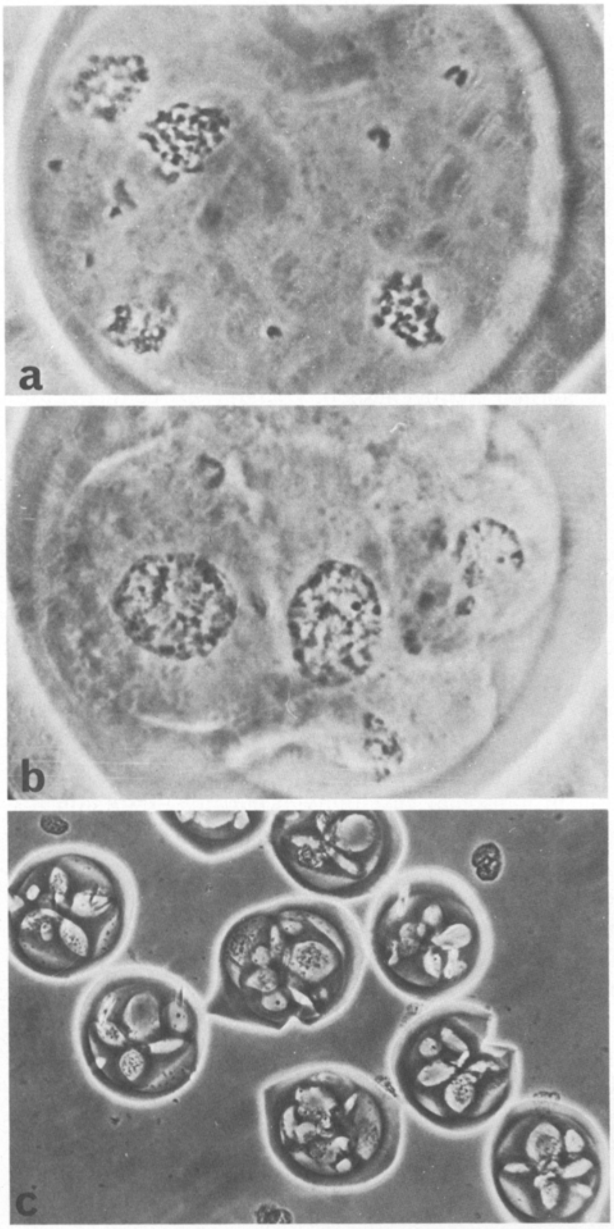

Fig. 2. **a** Anaphase-telophase. Single halve bivalents still condensed, other chromosome groups in telophase (× 1,260); **b** Each chromatin mass becomes isolated in a cell (cytokinesis) (× 1,260); **c** The sporad stage (× 240)

Figure 2a is a anaphase – telophase I. All the nuclei are not visible on this plate, but we can see a desynchronism in the chromatin cycle, single chromosomes are still condensed. The chromosome complement is separated here into 12 groups in the first meiotic division. Afterwards each chromatin mass becomes isolated in a cell (Fig. 2b) and does not undertake the second meiotic division. This pattern is general. Occasionally, when only four or five nuclei are formed at AI, then only the second meiotic division is observed. More rarely, one giant microspore is formed per sporad, giving a multipored pollen grain.

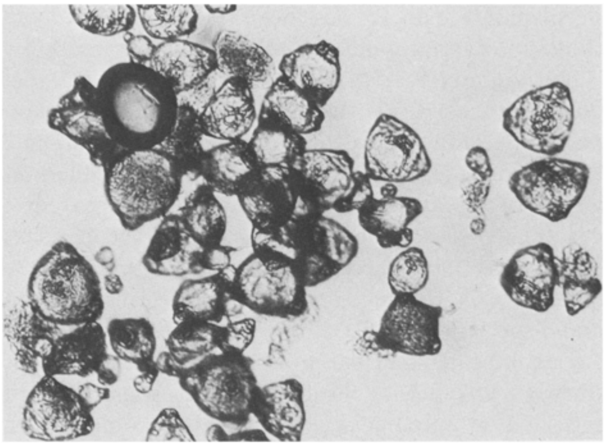

Fig. 3. Polymorphism of pollen grains at anthesis (× 120)

Figure 2c shows the callosic matrix at the sporad stage and the generalized pattern of microsporogenesis.

Polymorphism of the pollen grains at anthesis is illustrated by Fig. 3, in which we can see little unipored pollen grains, and bi-, tri-, tetra- and pentapored ones.

Discussion

According to John and Lewis (1975), meiosis is a mechanism which, in conjunction with fertilization, regulates, amongst other things, the chromosome number of an organism. A second feature of the meiotic mechanism is a redistribution of genetic material into daughter nuclei which is made possible by the segregational and recombination phases of the sequence. Only a part of the second feature may be assumed by this kind of division: segregation and partly, recombination. In the absence of the second meiotic division, (non-disjunction), the formed nuclei contain two copies of each chromosome that it has received and the resulting spores are "dianeuploid" and may be chimeric.

The term "complement fractionation" proposed by Thompson (1962) is the most judicious and obviously useful for the general phenomenon wherein the chromosome complement is subdivided into independently operating groups within a cell, the result being formation of gametes containing varying chromosomal complements.

Since, in vitro pollen germination has demonstrated that some pollen grains (3%) are viable, a novel source of variability is available with important genetical and cytogenetical changes (aneuploidy and reduction of the polyploidy level).

Wild fuchsias are diploids (2n = 22) and only two species are tetraploids. Horticultural fuchsias are mostly high-numbered polyploids including octaploids and decaploids (North 1979). The cv 'Riccartonia' is thus the most polyploid known (2n = c.140 = 12–13 x).

Polyploidy per se has been mentioned as contributing to chromosomal instability although instability is known in diploids (Vaarama 1949; Tai 1970; Carapetian and Rupert 1977). With increasing levels of autopolyploidy, lability was found to increase (Gottschalk 1959). For Tai (1970), plants with meiotic irregularities due to polyploidy and wide hybridization appeared to be specially susceptible to multipolar divisions. This view is the more acceptable. Indeed, the genome separation of chromosomes has been observed in diploid species hybrids.

This indicates that the grouping of chromosomes is somewhat linked with the formation of spindle fibers, suggesting a spindle organizer per genome. Thus, heterozygous spindle organizers exist in hybrid species or may exist in a species and, if they are unable to fuse or to coordinate their activities, this leads to multipolar or multiple spindle.

In the same way, high polyploids have several spindle organizers which at a determined level are unable to organize MI.

Such a situation "hybrid-highpolyploid" is that of Fuchsia cv 'Riccartonia. Thompson (1962) suggested that multipolar division provided a method of decreasing the level of ploidy. It may be possible that this cultivar has exceeded its bearable ploidy level. Polyploidy could thus be a reversible process. Diploid fuchsias have bipored pollen grains, tetraploids have tripored pollen grains, synthetic triploids have bipored pollen grains and some uni- and tripored ones.

Therefore, a correlation exists between the level of ploidy and the number of pores. Such a correlation seems to be verified amongst the *Onagraceae.* Unipored pollen grains are seemingly hypohaploids (sometimes only two chromosomes – the two chromatids of an anaphasic chromosome) which are empty before first pollen mitosis (the haploid chromosome set must be regarded as the smallest possible unit which renders an individual cell viable) while the others do not stop their internal differentiation. The in vitro germination is not linked with the number of pores. This seems to indicate that randomly formed chromosome groups are subject to a strong selection with the consequence that only the suitable gene combinations are able to form viable gametophytes and thus viable superreduced gametes.

As hoped by Thompson, who said: "it seems imperative to discover by examination of the other species, the universality of this cytological mechanism in order to determine its full evolutionary value and to find means of increasing its frequency so that it will provide a more useful tool for experimentalists."

In plants reproducing by sexual means, genes for complement fractionation are rapidly eliminated so that such irregularities are rare and sporadic in nature, but in vegetatively propagated plant, they are maintained and they are expressed under a generalized and uniform way. Horticultural fuchsias are a good tool for studying this phenomenon, by the use of colchicine-polyploidization, and for testing the potential value to plant breeding of complement fractionation by hybridization.

One of the characteristics that distinguishes cv 'Riccartonia' is the regular absence of the second division. Thompson, who carefully observed multiple spindles, noted that irregular sporads had 2–12 spores with a most frequent deviant type with 6 after the two meiotic divisions. In our case, the most frequent type of sporad has 9 microspores directly after the first division. Cellularisation occurs directly at TI while in the normal sporogenesis of *Onagraceae,* cellularization occurs after TII. The rare occurrence of the second division seems to indicate that the determining factor was the number of nuclei after the first division and, the spatial possibility of organizing the second division.

Another characteristic of our case is that the microspindles include bivalents instead of univalents as reported by Beadle and McClintock (1928) following an asynapsis. The figure reported by these authors is similar to some metaphases that we have observed in high polyploids of *Hymenophyllaceae* and that we have called "dispersional metaphases" (Tilquin 1981). This aberrant type is linked with apomixis.

Chromosomal instability occurring in somatic tissues has been clearly demonstrated by Vaarama (1949), and many others, and in meiotic cells, but not in both types of cells simultaneously. This seems confirmed in our case, because, if chromosomal instability occurred in somatic divisions, the cultivar could not be maintained.

Finally, we have seen that multipolar and multiple spindles exist side by side in the same cell and thus, for us, it is the same thing. As the literature is somewhat confused on this point, we believe that the better term is multiple spindle.

Ultrastructural aspects of this abnormal sporogenesis will be published elsewhere.

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