

U-type exchanges in *Muscari comosum* L. (Liliaceae)

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Some meiotic abnormalities, such as univalent formation, side-arm bridges, and bridges with fragments, were observed during both meiotic divisions in some individuals of *Muscari comosum* L. from one Spanish population. The latter aberrations were also detected during mitotic divisions. The majority of these abnormalities involved the first long pair of chromosomes. The exact nature of sister chromatid bridges has still not been elucidated. U-type exchanges can be explained on the basis of abnormal breakage and reunion occurring in the euchromatin that is near the heterochromatin present in the interstitial region of the long arm of the first bivalent. In accordance with the last aspect, there is no correlation between the distribution pattern of chiasmata within the first bivalent and the meiotic U-type exchanges. Spontaneous breakage and reunion events produce new combinations of chromosome segments in the first pair of chromosomes of *M. comosum*, and hence have been important from an evolutionary point of view in the origin of chromosome polymorphisms in this plant species.

Key words: *Muscari comosum*, meiosis, side-arm bridges, U-type exchanges, chiasmata distribution.

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Certaines anomalies méiotiques, comme la formation d'univalents, de ponts latéraux de bras de chromosomes et de ponts constitués de fragments chromosomiques, ont été observés au cours des deux divisions de la méiose et même, en ce qui concerne les dernières aberrations, celles-ci ont été décelées dans les divisions mitotiques chez certains individus de *Muscari comosum* L. d'une population d'Espagne. La majorité de ces anomalies ont impliqué la première longue paire de chromosomes. La nature exacte des ponts entre chromatides d'une même paire n'a pas encore été élucidée. Toutefois, les échanges de type U peuvent être expliqués par des bris anormaux suivis de leur réunion dans l'euchromatine qui est localisée près de l'hétérochromatine dans la région interstitielle du bras long du premier bivalent. Ceci est appuyé par le fait qu'il n'y a pas de corrélation entre la pattern de distribution des chiasmata à l'intérieur du premier bivalent et les échanges méiotiques de type U. Les bris spontanés, et par la suite leur réunion, produisent de nouvelles combinaisons de segments chez la première paire de chromosomes de *M. comosum*, ce qui a dû jouer un rôle d'un point de vue évolutif dans l'origine des polymorphismes chromosomiques chez cette espèce végétale.

Mots clés : *Muscari comosum*, méiose, ponts latéraux de bras chromosomiques, échanges en forme de U, distribution des chiasmata.

[Traduit par la revue]

Introduction

The spontaneous appearance of dicentric and acentric fragments has been observed during both meiotic divisions as well as during mitosis in both plant and animal species (Brandham 1969, 1970; Couzin and Fox 1973; Viinikka 1977; Karp and Jones 1983; Lewis and John 1966). Classically, the origin of dicentric and acentric fragments has been attributed to the occurrence of crossing-over within a paracentric inversion loop (McClintock 1931). However, such an origin requires several conditions to be met (Couzin and Fox 1962), and many cases are at variance with this hypothesis. Lewis and John (1966) suggested an alternative explanation for the formation of bridges and fragments. They proposed that there is a causal connection between the bridges and fragments at meiosis and chiasmata abnormalities. Such aberrant exchanges must be of the "U" type and involve sister chromatids in a bivalent. Evidence supporting this second hypothesis comes from the demonstration, in some cases, of a correlation between the distribution of chiasmata and the lengths of the fragments (Brandham 1970; Karp and Jones 1983).

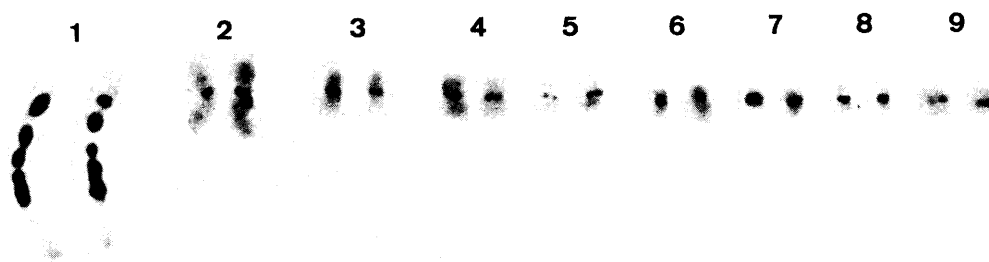
One question to consider is whether specialized chromosomal regions exist that are particularly sensitive to natural breakage and the formation of bridges with fragments. On the basis of observations made in several plant and animal species concerning the relationship between chiasmata and hetero-

chromatin (Jones 1978; Loidl 1982), another question can be posed: Are the chiasmata formed in the regions immediately adjacent to heterochromatin also sensitive to natural breakage? In *Najas marina*, Viinikka (1977) observed that the centromeric and interstitial dark C-bands in several chromosomes are especially prone to U-type exchanges.

In an attempt to answer these questions we have cytogenetically analyzed a liliaceous plant species, *Muscari comosum*, that expresses a characteristic C-banding pattern (Bentzer and Landstrom 1975; Ruiz Rejon et al. 1987). Furthermore, the occurrence of spontaneous fragments has been observed in this species (Bentzer 1972). In this paper we have attempted to answer these two questions, using this species with heterochromatic bands in only one chromosome pair. Specifically, (i) is this bivalent especially sensitive to U-type exchanges? and (ii) are the chiasmata that occur near the C-banding regions in this bivalent particularly sensitive to such types of phenomena?

Materials and methods

Anthers from 30 individuals of *M. comosum* L. from Silleta (Sierra Nevada, Granada, Spain) were analyzed. They were fixed in 3:1 ethanol – acetic acid and kept at 4°C for a minimum of 2 weeks. Slides of the stages of meiosis were prepared from pollen mother cells (PMC) by squashing in 2% acetocarmine. Three of these individuals

FIG. 1. C-banding pattern in *M. comosum*.

that had high frequencies of meiotic anomalies were analyzed simultaneously with C-banding methods to determine the exact locations of chiasmata within the first bivalent (in the heterochromatic or euchromatic chromosome zone), and the amount of heterochromatin present in the fragments was observed at anaphase I. Chiasmata were classified as proximal, interstitial, and distal in relation to the centromere. Fragment sizes were measured using all of the cells with bridge and fragment configurations from each individual. The fragments were grouped into three classes of equal size interval and designated as small, medium, or large. Slides whose chromosomes were Giemsa C-banded were prepared according to Schwarzscher et al. (1980) with minor modifications. Mitotic preparations were obtained from root tip meristems that were squashed in acetoorcein.

Results

Cytogenetic characteristics of *M. comosum*

This species has a karyotype with $2n = 18$ chromosomes that includes one long pair of subtelocentric chromosomes, one pair of intermediate size metacentric, submetacentric, or subtelocentric chromosomes (because of two stable chromosomal polymorphisms for a pericentric inversion and probably for an unequal interchange or an insertion translocation (see Ruiz Rejon et al. 1987)), and seven pairs of small metacentric chromosomes (Fig. 1). In C-banding preparations the long arm of the first pair of chromosomes shows from three to eight bands localized in the interstitial position (Fig. 1; Bentzer and Landstrom 1975).

Disturbances in cell divisions

In most meiocytes, zygotene pairing was complete and nine bivalents were observed during diplotene. In some meiocytes at diplotene–diakinesis, univalents were observed. The majority of these involved homologues of the first chromosome pair. However, in some cases, one of the small bivalents also showed this behaviour.

The most prominent feature during the first meiotic anaphase was the high frequency of chromosomal abnormalities. The most common aberration observed was the presence of bridges with and without fragments that were derived from the first bivalent.

Bridges without fragments almost invariably involved the long arm of the longest chromosome (Fig. 2). Two types of bridges were found: (i) the two side-arms appeared to diverge from a single point on the bridge (Fig. 2), and (ii) the side-arms were parallel and inserted slightly apart from each other (Fig. 3). The bridges most frequently seen were those that occurred in the interstitial region. However, bridges without fragments were also observed and involved the second



FIG. 2. Anaphase I with one bridge where the two side-arms appear to diverge from a single point. FIG. 3. Anaphase I with two bridges. The side-arms of the first pair are parallel and inserted apart from each other. Furthermore, one small bivalent shows a bridge.

chromosome pair (Fig. 3) and one of the smallest bivalents. The involvement of these bivalents in bridge and fragment formation was very low ($<1.0\%$).

Among the 30 plants in which spontaneous bridges were studied, the frequency of bridges without fragments ranged from 0 in two individuals to more than 20.87% in another individual, with a mean frequency of 13.75%.

With respect to the bridges with fragments observed at anaphase I that originated from the first bivalent, the most remarkable observation was that the size of the fragment varied in the same individual. Thus, we observe bridges with small (S) (Fig. 4), medium (M) (Fig. 5), or large (L) (Fig. 6) fragments. The frequency of aberrant cells with fragments ranged from 0 in six individuals to 11.76% in one individual, with a frequency mean of 2.00%.

Anaphase I bridges may persist through interkinesis and even into metaphase II cells. Bridges were common at anaphase II and occasionally had fragments associated with them. Whether such spontaneous chromosome aberrations are only associated with meiotic divisions was determined by examining root tip mitoses in individuals in which meiotic aberrations were detected. Mitosis was found to be very regular, although occasionally bridges without fragments were observed.

Correlation between chiasmata localization and size of fragments

In three individuals in which high frequencies of bridges with fragments were detected for the first bivalent, we attempted to determine if there was a correlation between the

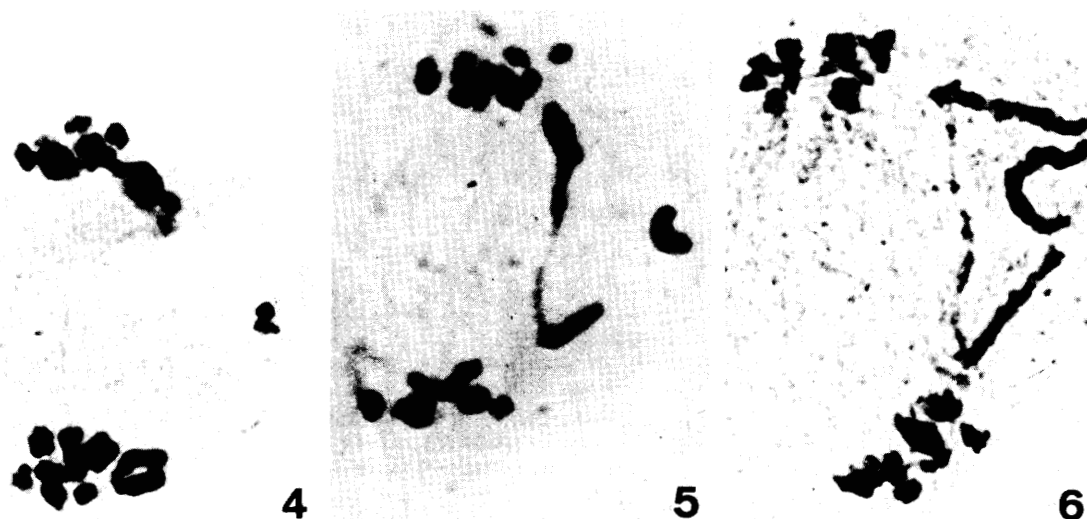


FIG. 4. Anaphase I cell showing a bridge with fragment of small length. FIG. 5. Anaphase I cell showing a bridge with fragment of medium length. FIG. 6. Anaphase I cell showing a bridge with fragment of large length.

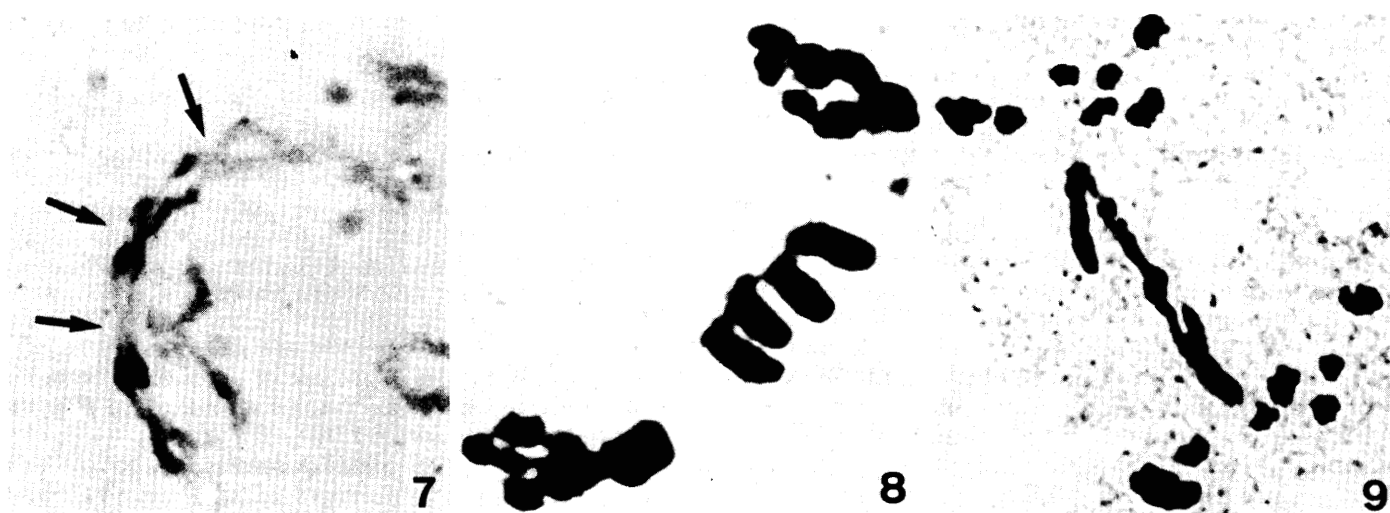


FIG. 7. C-banding of diplotene cell of *M. comosum*. FIG. 8. Anaphase I configurations presumably originated by a U-type exchange in the short arm of the first bivalent. FIG. 9. Anaphase I configurations that can be due both to sub-arm bridge or a U-type exchange in the terminal position of the long arm of the first bivalent.

patterns and frequencies of chiasmata distribution for this bivalent, and the positions and frequencies of occurrence of the several types of bridges with fragments, by observing C-banding patterns and distributions.

In the first bivalent there were five positions in which chiasmata occurred: terminal in the short arm (Fig. 7), proximal to the centromere in the long arm, and interstitial, distal, and terminal in the long arm.

The anomalous exchanges occurring in the short arm of the first bivalent produce the configuration shown in Fig. 8. The exchanges produced in the terminal positions of the long arm produce the configuration shown in Fig. 9. In our analysis we did not include the chiasmata frequencies in both terminal positions because their frequencies were very low. The frequency data of proximal, interstitial, and distal chiasmata from the three individuals sampled are given in Table 1. The results indicate that the three individuals show very similar chiasmata

frequencies and that the most frequent chiasmata are those produced in interstitial positions. These chiasmata always occur within euchromatic regions (Fig. 7). The fragments observed were classified into three groups of equal size intervals (in relation to the length of the long arm of first bivalent). Thus, from 10–40% were designated as small (S), from 40% to 70% as medium (M), and from 70 to 100% as large (L). We found that the medium size fragment was the most frequent (Table 2).

With respect to heterochromatin content of each type of fragment, we observed that the small ones did not contain heterochromatin (Fig. 10). The medium-sized fragments carried only a few heterochromatin segments (Fig. 11), while a high number of heterochromatic blocks were present in the large fragments (Fig. 12).

A correlation test indicated that no correlation exists between the chiasmata types and their corresponding fragment sizes ($r = 0.2936$, $t = 0.33$, $p < 0.05$). This lack of correla-

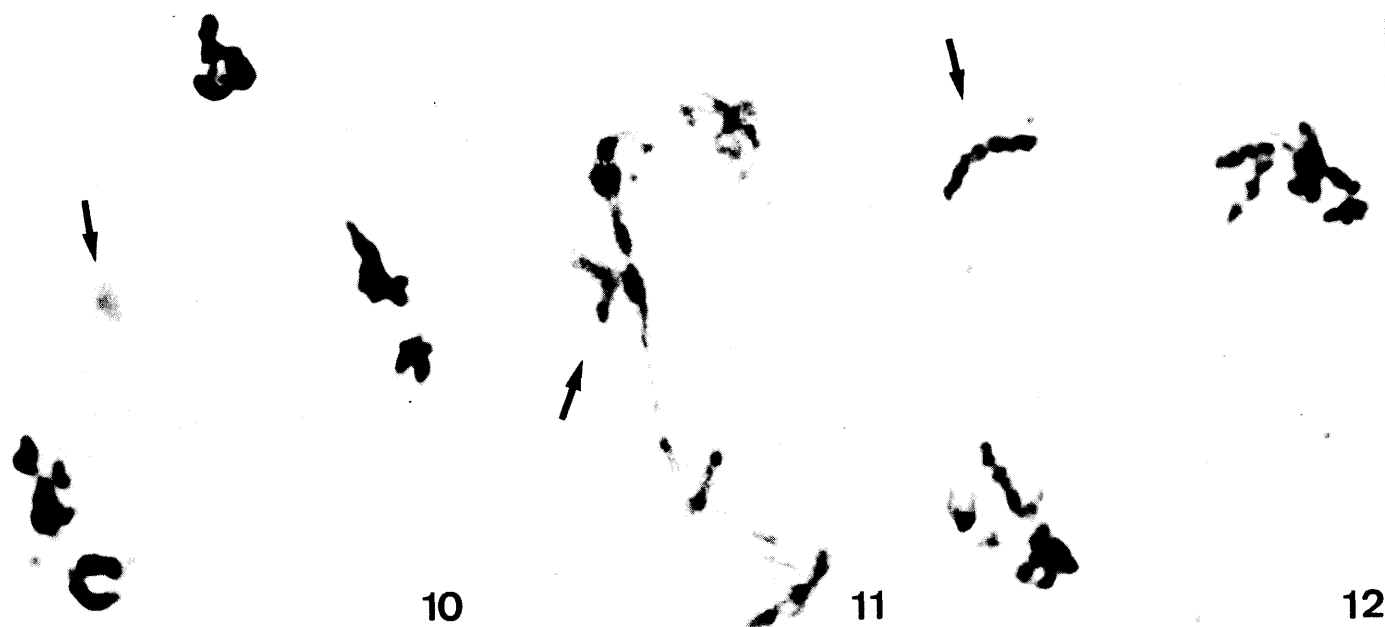


FIG. 10. C-banding of anaphase I cell with a small fragment. FIG. 11. C-banding of anaphase I cell with a medium fragment. FIG. 12. C-banding of anaphase I cell with a large fragment.

TABLE 1. Number of diakinesis cells with proximal (P), interstitial (I), and distal (D) chiasmata in the first chromosome pair (terminal chiasmata were not scored)

Individual	P	I	D	Total
Silleta 2	41	18	43	102
Silleta 22	46	28	61	135
Silleta 30	58	30	52	140

TABLE 2. Number of anaphase I cells with small (S), medium (M), and large (L) fragments in relation to the length of the long arm of the first chromosome pair

Individual	S	M	L	Total
Silleta 2	3	10	3	16
Silleta 22	4	15	6	25
Silleta 30	5	15	7	27

tion may be due to the fact that chiasmata are more frequent in the distal and proximal positions. On the contrary, the fragments produced in the interstitial position (M) are the most frequent.

Discussion

Bridges without fragments observed in *M. comosum* can be attributable to subchromatid exchanges or side-arm bridges. These phenomena have been reported in both plant and animal species (Brandham 1969, 1970; Gosalvez et al. 1981) and can have a spontaneous or induced origin. The causal factors suggested to describe their origin range from stickiness, failure of reproduction, and subchromatid exchanges, to prolongation

of pairing (John and Lewis 1965). However, the nature of these bridges is still unknown, although certain models, such as those based on half chromatid exchanges, can be excluded if the chromatid is unineme in structure, as is now generally accepted.

There are several observations that lead us to think that the bridges and fragments observed at anaphase I in *M. comosum* are caused by U-type exchanges: (i) The size of bridges and fragments varies greatly in the same individual (Figs. 4, 5, and 6); (ii) The formation of precocious univalents indicates failure in the formation of chiasmata (Fig. 2); (iii) The bridges with fragments are also observed to occur in the second bivalent and in one of the smallest bivalents. This observation indicates that the synapsis failures also occur in bivalents other than the first one. In addition, the disturbances observed in mitotic divisions cannot be explained on the basis of the inversion theory.

The U-type exchanges appear in genetically unusual types, such as F_1 hybrids, cultivated inbred lines (Karp and Jones 1983), or species with a predominantly asexual mode of reproduction. In these cases, this abnormality has been explained as a part of the syndrome of errors reflecting a breakdown in the control of meiotic processes. However, Viinikka (1977) found U-type exchanges in some natural plants such as *Najas marina* and suggested that heterochromatic regions are particularly sensitive to breakage.

However, in *M. comosum*, U-type exchanges involved only one pair of chromosomes (the longest pair). This is different from *N. marina* in which U-type exchanges involved several chromosome pairs (I, II, IV, and V). After C-banding in *M. comosum*, from three to eight C-bands were visible in the long arm of the first pair of chromosomes (Fig. 1) (Bentzer and Landstrom 1975), and in *N. marina* the four chromosomes mentioned above were observed to possess interstitial C-bands. It seems that in these two natural species with U-type exchanges, chromosomes that have interstitial C-band regions may be particularly prone to U-type exchanges. Furthermore, in the case of *M. comosum*, the U-type exchanges most fre-

quently observed are those that occur in the interstitial region of the long arm of the first chromosome pair. The majority of the three to eight bands of *M. comosum* are localized in this interstitial position of the first chromosome. Likewise, in this interstitial region the side-arm bridges occur most frequently. This indicates that chiasmata formation close to interstitial C-bands are particularly prone to errors, at least in some plant species.

The possible cause of errors in chiasmata formation in these heterochromatin-rich regions is explained in the following. Since the main features of the molecular mechanism of recombination are now generally accepted (Holliday 1974), i.e., nicks in the DNA, heteroduplex formation, and repair synthesis, the production of aberrations in chiasmata formation is most probably because of the errors in the repair synthesis, nicks not being identified, or being corrected erroneously. But DNA nicking is not randomly distributed. In the mouse, for example, the 400–500 repetitive sequences and foldbacks are the principal targets of error in repair synthesis, and satellite DNA is totally void of them (Hotta and Stern 1978). Therefore, DNA nicking could be particularly frequent in chromosome regions very rich in these repetitive sequences. On the basis of studies in *Allium* species, Loidl (1982) claimed that certain repetitive sequences could constitute marginal parts of some C-bands. If this is true in *M. comosum* (and in *N. marina*), it is possible that next to the interstitial C-bands there exist certain repetitive sequences that act as efficient targets for DNA nicking necessary for chiasma formation. This nonrandom DNA nicking distribution raises the probability that nicks close to C-bands are not being identified or are being corrected erroneously, and consequently they could give rise to U-type or side-arm bridges exchanges. In fact, in *M. comosum* the chiasmata present in the interstitial regions of the first bivalent always occur in euchromatic zones placed next to heterochromatic ones (see Fig. 7). Furthermore, the most frequent fragments are the medium-sized ones, and they are produced by anomalous exchanges in interstitial positions where several C-bands exist.

On the other hand, the extensive occurrence of U-type exchanges in the long arm of the first chromosome pair of *M. comosum* can explain the existence of an extensive polymorphism in relation to the number and position of C-bands of this chromosome detected by Bentzer and Landstrom (1975). This array of new combinations (with variable number of C-bands) may be of selective value, and new karyotypes may arise.

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