Heredity (1978), **41** (1), 101-103

NOTES AND COMMENTS

DIFFERENTIAL GIEMSA STAINING OF B CHROMOSOMES OF *FRITILLARIA TENELLA*

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Received 15.ii.78

Summary

Ten telocentric B chromosomes, identical in size and banding pattern have been observed at mitosis in root tips of a plant of *Fritillaria tenella* ($2n = 18$), prepared and stained by Giemsa to show C-banding. It is proposed that, following formation of an initial B by misdivision at one side of the centromere in a sub-terminal univalent chromosome at meiosis in an ancestral plant, the Bs have multiplied with successive generations of breeding. It is assumed that this occurred by post-meiotic non-disjunction either in pollen or embryo sac, so as to produce gametes with double the number of Bs. The centromeric structure of the Bs was complex and sometimes appeared to consist of a single innermost dot contiguous with a tiny terminal bipartite C-band. The supposition is made that the single dot is one of the two dots representing the centromere, the other sometimes being orientated out of view. Further heterochromatin was present in the Bs as shown by the presence of a prominent C-band at the telomeric end. All the A chromosomes showed C-bands, with as many as 12 in one M. Heteromorphy in respect of bands was evident in an M chromosome.

1. Introduction

During a study of C-banding in mitotic chromosomes of several species of *Fritillaria*, the complement of a plant of *F. tenella* was found to contain supernumerary B chromosomes. The present paper is largely concerned with the structure of the centromere in the Bs, as revealed by differential Giemsa staining to show constitutive heterochromatin.

2. Materials and methods

Root tips from potted plants were treated with colchicine 0·05 per cent (3-4 hours) and fixed in ethanol/glacial acetic acid (3 : 1) overnight. They were then prepared as squashes for application of the C-banding technique, using the schedule described by Darlington and La Cour (1976).

3. Observations and discussion

F. tenella is a diploid ($2n = 18$) and is, I believe, indistinguishable from *F. ruthenica* with the same chromosome number. Both have similar complements, comprised of five pairs of M (metacentric) chromosomes and four pairs of STs (sub-terminal).

Most other fritillaries have $2n = 24$ chromosomes, consisting of two pairs of Ms and 10 pairs of STs. Darlington (1937) considered that the reduced basic number in *F. ruthenica* arose from changes in a plant with $2n = 24$, leading to an increase in number of Ms by fusion of rods (STs).

The plant of *F. tenella* used for study of C-banding had 10 B chromosomes (plate 1, fig. 1A). These were all clearly identical, as indicated by equality of size and banding pattern. The absence of a short arm indicated that they were telocentrics. They were remarkable, however, in that, as was clear in well stained Giemsa preparations and with high magnification, they appeared to have a compound centromeric structure. When most clearly seen this appeared to be tripartite (plate 1, fig. 1B).

A prominent C-band was present at their telomeric end. C-bands were present in all other chromosomes, some being close to the limits of resolution. One of the Ms contained as many as 12 bands. The largest was confined to a single M chromosome, thus indicating heteromorphy in one M pair. Most bands were clearly bipartite reflecting the presence of chromatids.

Pairs of centromeric dots, representing the functional part of centromeric structure (Marks, 1977), were distinguishable in some chromosomes, but sometimes in others instead of pairs of dots only a single one was present within the constriction. Plate 1, fig. 1B shows both situations clearly and that which is seen in individual chromosomes most probably depends on orientation of the centromere relative to the plane of observation.

Their identical size, banding and centromeric structure provide an indication as to the origin of the Bs. It seems certain that, following the initial formation of a single B in an ancestral plant, the Bs have multiplied with successive generations of breeding. This has most probably come about by post-meiotic non-disjunction either in pollen and/or embryo sac, so as to produce male and female gametes with double the number of Bs, as already found in other plants (*e.g.* Müntzing, 1945, 1946; Håkansson, 1948; Battaglia, 1964). We have no knowledge as to whether in *Fritillaria* such non-disjunction occurs in both pollen and embryo sac. Apparently in certain species of lilies non-disjunction of Bs is limited to the embryo sac (Müntzing, 1967) and in *Lolium perenne* the male side (Rees, 1974).

The telocentric nature of the Bs suggest that it is highly probable that the original B arose at meiosis by misdivision of the centromere in an ST univalent chromosome of a trisomic plant. Such misdivision is common in some *Fritillarias* and it was in a member of this genus that misdivision was first described by Darlington (1939). It was not possible, however, to recognise the chromosome from which it was derived.

It is open to question as to what part of the tripartite centromeric structure seen in the Bs represents the centromere. Before attempting to answer this question, however, it is necessary to make certain points clear. First, it seems correct to assume that the tripartite structure observed is not the complete one. Observation was hampered by the fact that divisions were few and, because of the structures size, visualisation was highly dependent on maximum Giemsa staining.

It can be expected that all C-bands which traverse most of the diameter of the chromatids will appear as bipartite structures, when the chromatids lie in the same or approximately similar plane. An exception occurs when the bands completely span the chromatids, so as to appear as a large solid band, as evident in one of the M chromosomes and in the telomeric band of the Bs (plate 1, fig. 1B). On the other hand the centromere, as we have seen, comprised of a pair of dots, as indicated by Eiberg (1974) and confirmed by Marks (1977), can be orientated so as to appear as a single dot according to the plane of viewing.

Plate 1

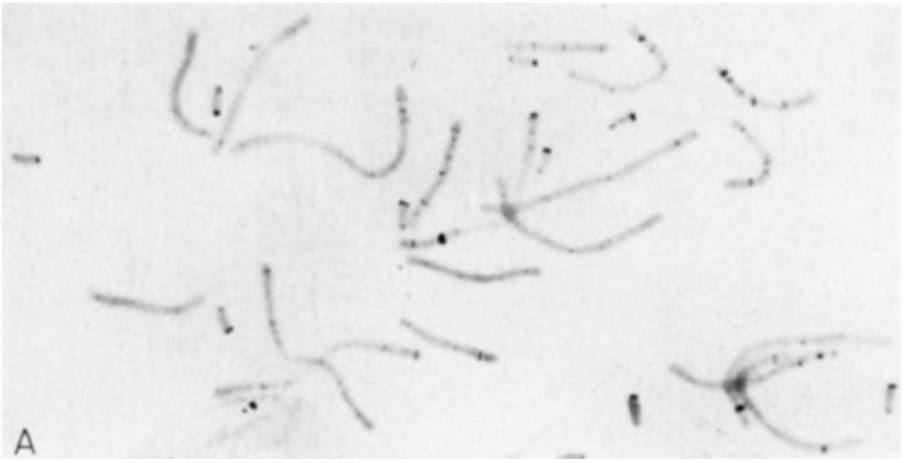

FIG. 1A.—Metaphase from a root tip of a plant of *Fritillaria tenella* ($2n = 18$) with an additional 10B-chromosomes, differentially stained by a Giemsa technique to show C-bands. × 1600.

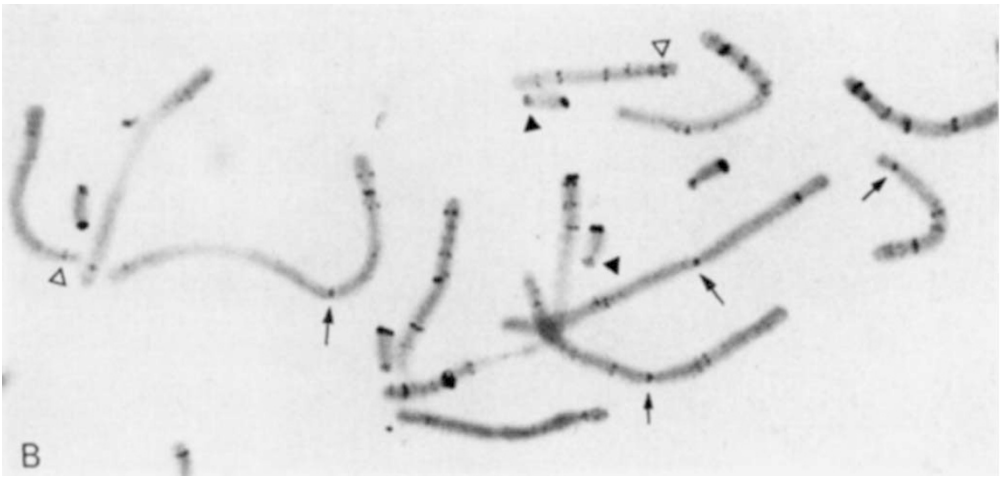

FIG. 1B.—Shows some of the banded chromosomes at higher magnification. Closed arrow heads indicate tripartite appearance of centromeric regions in the Bs, open arrow heads the pairs of centromeric dots (representing centromeres) and arrows the centric constrictions in some M chromosomes in which, because of orientation, only one such dot is seen. × 2800.

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It therefore seems probable that such an orientation is responsible for the tripartite structure seen in the Bs. If this interpretation is correct, the actual centromeric structure of these chromosomes most probably consists of the functional centromere, lying innermost and comprised of two dots, and contiguous with them additional heterochromatin representing a terminal bipartite band. It is perhaps more than by chance that Bs with the complete implied structure were not seen. It may therefore be open to question as to whether the immediately adjacent C-band interferes with orientation of the centromere.

The implication that these telocentrics have pairs of centromeric dots similar to those in the A chromosomes, suggest that breakage (misdivision) giving rise to the first B occurred at one side of the dots. It is relevant to note that in respect of two telocentrics found in *Nigella doerfleri* by Marks (1977), both had pairs of centromeric dots similar to those of their SM homologue from which they originated.

The complete centromeric region (the constriction) between the chromosome arms, in which the centromere itself has a central position, is said to be always symmetrical in respect of chromomeric structure and have essentially the form of a tandem reversed repeat (Lima-De Faria, 1956). If the present interpretation of the centromeric structure of the Bs in *F. tenella* is correct, then it seems clear that Lima-De Faria's model could not apply to the A chromosome from which the initial B was derived, since the breakage must have occurred beyond or within the immediately adjacent heterochromatin that is confined to one side of the pair of centromeric dots. This is far from being improbable since in a recent study involving C-bands in other fritillaries additional heterochromatin has been found fairly frequently within the centric constriction, presumably immediately adjacent to the centromeric dots which are then excluded from view (La Cour, 1978). This is not to be confused with the more common situation in which heterochromatin may lie adjacent but outside the constriction.

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