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The B Chromosome System of *Scilla autumnalis* (Liliaceae) : Effects at the Isozyme Level

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Abstract. Supernumerary (B) chromosomes have been studied in a Spanish population of Scilla *autumnalis* L. (Liliaceae). Out of the 140 individuals analysed, seven had 2n=14+lB, one 2n=l4+2B, one 2n=14+3B and one 2n= 14 + 9B. An analysis of esterase isozyme patterns shows that all 130 individuals with a standard karyotype (2n= 14) have two esterase loci, Est-2 and Est-3. whereas all 10 individuals with Bs have three, Est-1, Est-2 and Est-3, irrespective of the precise number of Bs present. The role that the Bs may have played in the appearance of this new locus (Est-1) is discussed in relation to their possible origin.

Introduction

Supernumerary or B-chromosomes have been identified by Battaglia (1963, 1964) in single plants of *Scilla autumnalis* (2n= 14) from Sicily (2n= 14+3B) and Palestine (2n= 14+&8B). We have also found them in material of this species from the Iberian Peninsula. Thus in a population from Torre de1 Vinagre (Sierra de Cazorla, Jaen) there are individuals with 1, 2, 3 and 9B chromosomes respectively (Ruiz Rejon and Oliver. 1978).

In this paper we report on a consistent correlation between the presence of B-chromosomes and the activity of a distinctive esterase isozyme.

Materials and Methods

In all. 140 bulbs of Scilla uutun~nalis L. were collected from Torre del Vinagre and examined cytologically.

Bearing in mind that S~~ilk~ can reproduce vegetativelly the bulbs were usually collected more than one meter apart.

Mitotic analysis was carried out on meristems of root tips squashed in acetic-orcein. Meiotic behaviour of the PMCs was analysed by staining young anthers in acetic-carmme. Esterase isozymes, present in crude extracts obtained after crushing roots. leaves and flowers. were studied by horirontal starch gel electrophoresis (see Oliver and Ruiz Rejon. 1980: Ruir Rejon and Oliver. 1980).

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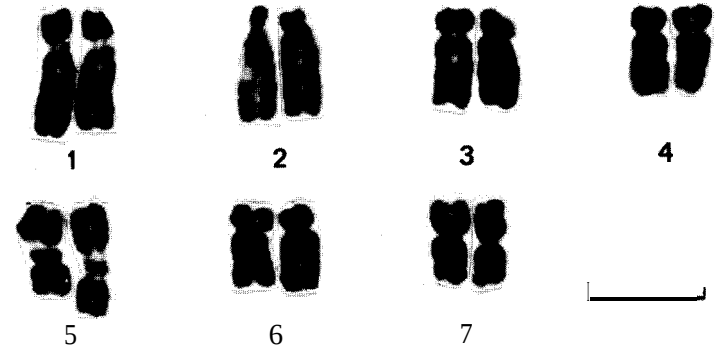
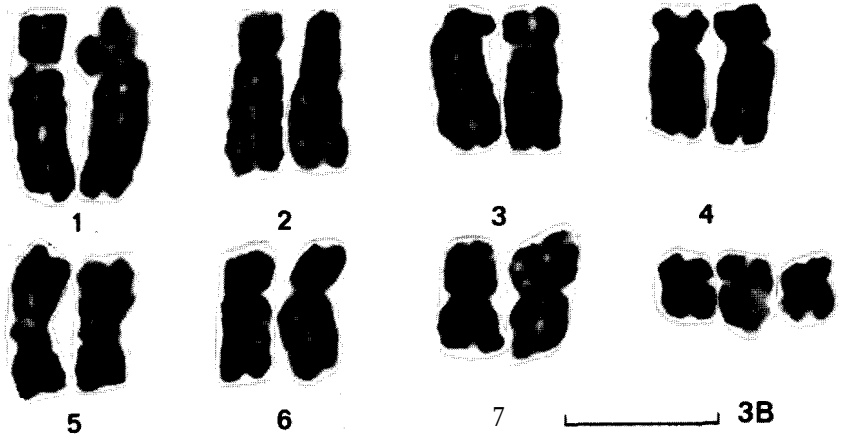
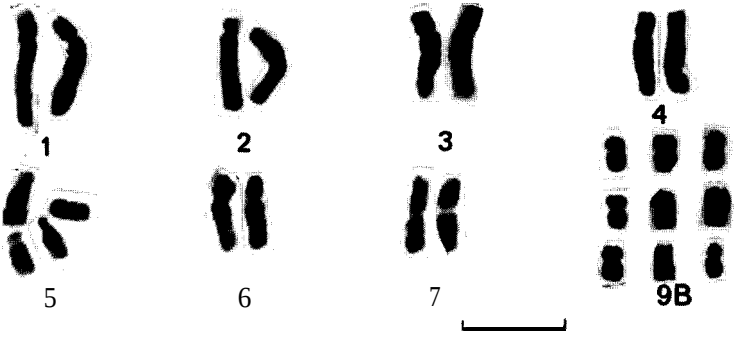






Figs. l-3. Standard karyotype and B-chromosome structure in *Scilla autumnalis* L. Fig. **1.** Standard complement with 2n = 14. Figs. 2 and 3. Karyotypcs from individuals with three (2n = 14 + 3B) and nine supernumeraries (2n = 14 + 9B). respectively. Bars = 5 μm

Results

1. Karyotypes

Of the 140 bulbs studied 130 had a standard karyotype (Fig. 1) including 4 pairs (1–4) of acrocentric chromosomes, one metacentric pair with an intercalary satellite (5) and two smaller pairs one of which was acrocentric (6) with the other a metacentric (7).

In 7 individuals one extra metacentric chromosome (B or accessory) was present together with the 14 members of the normal complement. This supernumerary showed the same staining property as the A chromosomes when condensed though it was smaller than them.

In three further individuals there were respectively two. three (Fig. 2) and nine (Fig. 3) B chromosomes present. In all 10 cases the Bs were uniform in size and morphology.

No chromocentres of any kind were observed in the interphase nuclei of individuals without accessory chromosomes. Where Bs were present there were an equivalent number of heteropycnotic bodies in resting nuclei (Fig. 4).

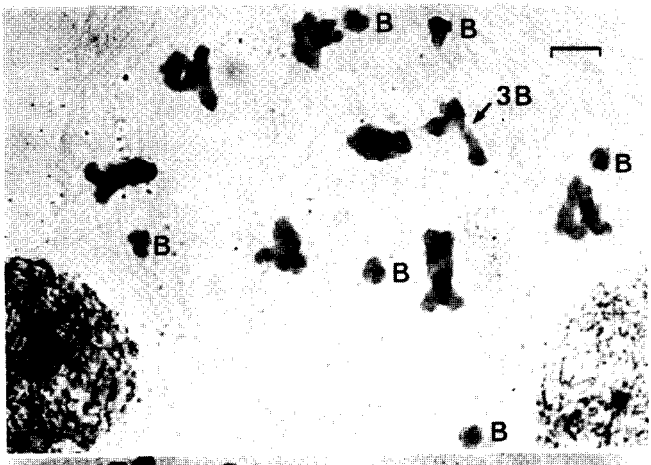
2. *Meiotic Behaviou*

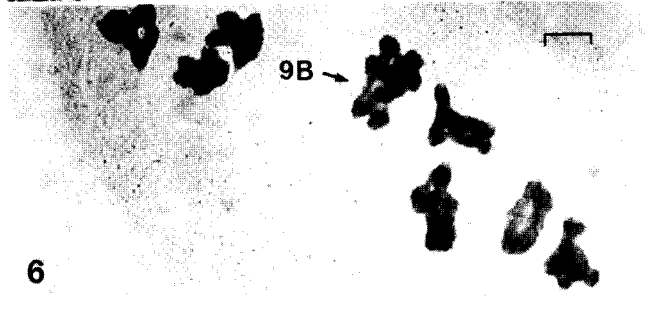
Meiotic behaviour was regular in the case of the individuals without supernumeraries, except for the occasional presence of univalents. Individuals with 9B chromosomes. on the other hand, show an average of 3–4 accessory univalents in every PMC (min. 0, max. 6) with the remaining Bs forming bivalents (1–2), trivalents (0–1), a pentavalent (O-l) or else agglutination figures involving all 9 Bs (Figs. 5, 6). Sticky associations were also common between the B and A chromosomes (Fig. 7).

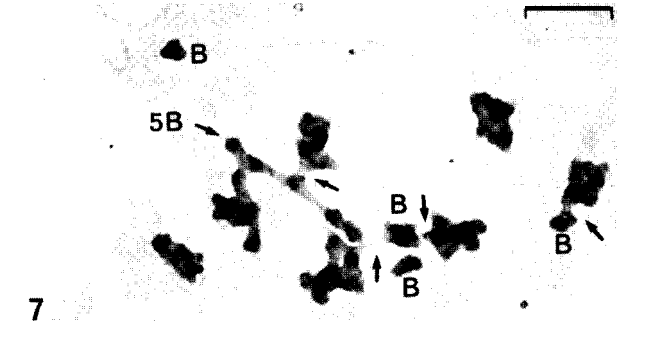
3. *Esterase Variability*

A total of ten esterase electromorphs can be detected by means of starch gel electrophoresis in individuals from the Torre del Vinagre population. The pheno-

Fig. 4. Interphase nuclei from an individual with 2 Bs. Notice the presence of two chromocentres (*arrows*). Bar = IO μm







Figs. 5-7. Metaphase-I pairing among the supernumeraries in the individual with 9 Bs. Fig. 5. Cell with one trivalent (*arrow*) and six univalent accessories. Fig.6. Cell with an agglutination figure including all nine Bs (*arrow*). Fig.7. Cell with a B pentavalent chain (*arrow*) and four B univalents. Note the presence of numerous sticky associations between the A and B-chromosomes (*arrows*). Bar= 5 μm

typic distribution of these electrophoretic bands among the 140 individuals from this population. and the data from 404 other individuals belonging to seven other natural populations of this species that have separately been analysed (Posse et al. 1980), allow us to group all the electrophoretic bands in three esterase activity zones. With decreasing mobility towards the anode these are

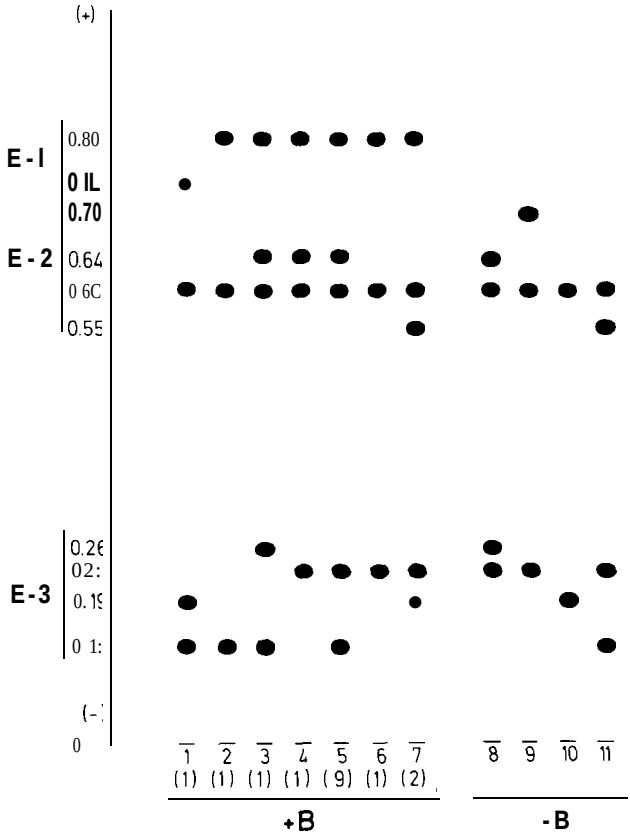


Fig. 8. Esterase isozyme patterns in individuals with (samples 1–7) and without Bs (samples 8-l 1). The precise number of Bs is indicated in brackets. The variant bands are defined according to their relative migration. The origin is indicated by 0

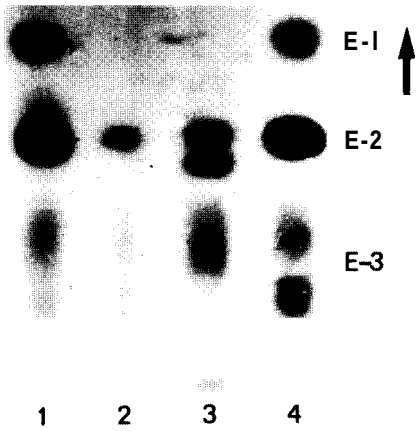


Fig. 9. Electrophoretic phenotypes of esterases in *Scilla autumnalis*. 1 and 4 individuals with 1 and 3 Bs respectively. 2 and 3 individuals without Bs

E-l, E-2 and E-3, with two, four and four allelic variants respectively (Fig. 8). In addition, a "null" allele is known to be present in the E-2 zone. though it has not been detected in the Torre del Vinagre population. The expression of Est-1, Est-2 and Est-3 genes is the same irrespective of the plant tissue analysed. However, the appearance on the gels of the E-l zone varies among the different individuals from the Torre del Vinagre population (Figs. 8 and 9). The bands of the E-l zone appear exclusively in the ten individuals carrying supernumerary chromosomes (Fig. 8). Furthermore, Bs have not been found in individuals where the E-l zone of esterase activity has not been found whereas the E-2 and E-3 zones appear in all the individuals from this population (Fig. 9).

Discussion

From previous investigations it is known that while B-chromosomes rarely influence discontinuous aspects of the phenotype, they commonly affect the expression of quantitative characters.

Thus a number of authors have detected an influence of Bs on chiasma frequency, the viability of the pollen, gametes. seeds and embryos, fertility, rate of development. the weight and protein content in the seeds and several other quantitative characters.

At the nuclear level, Ayonoadu and Rees (1968) in *Secale* and Barlow (1973) in *Puschkinia libanotica* have shown that the presence of the Bs increases the duration of the mitotic cycle in the radicle meristem cells. Several authors (Himes, 1967 ; Jones and Rees, 1968 and Kirk and Jones, 1970) have also demonstrated that Bs affect the histone/DNA ratio of the nucleus as well as RNA synthesis and A-chromosome volume. All these findings argue that B-chromosomes may exert regulatory effects on the nucleus, modifying or suppressing gene action. If this is the case then the synthesis of proteins or enzymes could also be affected by their presence. Indeed, Bergerard et al. (1972) have already claimed an influence of supernumerary chromosomes on the hemolymph proteins of *Locusta migratoria.*

Our observation that an additional esterase isozyme (E-l) appears in individuals of S. *autumnalis* with Bs suggests that its appearance is determined by the presence of the supernumerary. Alternatively, one could postulate that the appearance of the isozyme E-l is independent of the presence of Bs which implies that a "null" allele of the Est-1 locus is fixed in the individuals without Bs. This would be more likely if the individuals with and without Bs were confined to distinct clonal samples. The electromorph variability at E-2 and E-3 zones, within both groups of individuals (see Fig. 8), makes such a hypothesis untenable. Moreover, the consistency of the relationship favours the hypothesis that the supernumerary chromosomes in this instance are indeed contributing to the molecular phenotype of those individuals that carry them.

Battaglia (1963, 1964) suggested a series of hypothetical rearrangements, during the meiotic process and at the level of the secondary constriction in the 5th chromosome pair. as a possible mechanism for the origin of the Bs in *Scilla autumnalis.* Although the scheme is not convincing to some workers

because the nucleolus organizer region is known to be capable of considerable extension both in the production of squash preparations and under natural circumstances (John, 1976) cases are certainly known where genuine breakage does occur at the secondary constriction (see, for example, Marchant and Brighton, 1971). Whether or not such a mechanism explains the origin of Bs in S. *autumnalis* there can be no question but that they must have arisen in some sense by partial duplication of A material. Consequently two hypotheses can be proposed to explain the possible role played by the accessory chromosomes in the origin of the isozyme E-l :

1) The structural gene specifying the isozyme E-l may be situated on the accessory chromosomes.

2) The Bs may carry a gene regulating expression of the structural gene for E-l.

In either case the gene situated on the accessory chromosomes, whether structural or regulatory, could have originated by the duplication of an ancestral gene from the A5 pair if, as Battaglia claims, this chromosome did sire the B. The appearance of a new isozyme could then be explained by evolutionary divergence through mutation and diversification, a frequent process where there is gene duplication (Ohno, 1970; Markert et al., 1975; Lim and Bailey, 1977; Ferris and Whitt, 1977; Garcia-Olmedo et al.. 1978), or by a position effect.

At the moment we lack direct evidence to choose one or other hypothesis, although the identical relative staining intensity of electrophoretic bands of zone E-l, irrespective of the number of Bs, indicates a lack of gene dosage response. This in turn argues against the presence of a structural gene and supports the presence of a regulatory effects.

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